

THE COMPOUND EYES OF THE ISOPODA
AND AMPHIPODA (CRUSTACEA)

MORPHOLOGY AND FUNCTION

HEIMO L NILSSON

Lund 1982



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THE COMPOUND EYES OF THE ISOPODA AND AMPHIPODA (CRUSTACEA)

Morphology and Function

av

Heimo L Nilsson

Fil kand, Hld

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Title and subtitle The Compound Eyes of the Isopoda and Amphipoda (Crustacea). Morphology and Function			
Abstract Problems: 1: Contemporary knowledge of the ultra-structure of isopod and amphipod (Crustacea) compound eyes is limited. Thus, a detailed description of the ultrastructure and morphology of these eyes is presented to provide a solid basis for functional investigations and phylogenetic speculations. 2. The deep-water forms of isopods and amphipods possess eyes that are structurally similar, and they appear to be morphologically well-adapted to low light intensities. The central problem, then, is how these eyes are functionally adapted. Methods: Included were histology (light, transmission and scanning electron microscopy); optics (conventional light and interference microscopy); and sensitivity measurements (electroretinograms, ERG). Results: 1. The isopod eye exhibits a diversity of structural details, especially as regards the morphology of the rhabdoms, but also as regards the morphology of the dioptric elements. 2. The fine structure of the amphipod eye varies and four sub-types are described. All amphipods possess five retinula cells. 3. A number of features are homologues in the two suborders, e.g. the presence of a fenestrated membrane and the number of cells comprising the crystalline cones. Several cases of convergent development are present. 4. The eye of an isopod deep-water form exhibits an elaborate tapetal layer, large acceptance angles, a single spectral sensitivity peak, a high visual threshold sensitivity and a low temporal resolution. In this type of eye, retinal dystrophy is caused by excessive light.			
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ERRATA

The Compound Eyes of the Isopoda and Amphipoda (Crustacea). Morphology and Function

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From the Department of Zoology, University of Lund, Lund, Sweden

Heimo L. Nilsson

THE COMPOUND EYES OF THE ISOPODA AND AMPHIPODA (CRUSTACEA)

Morphology and Function

Lund 1982

For my family Annika and Johannes
and my parents Elin and Ragnar



Time obliterates the fictions of opinion
and confirms the decisions of nature

- Cicero in De Natura Deorum II 25

This thesis is based on the following papers:

- I. Nilsson, H. L.: The fine structure of the compound eyes of shallow-water asellotes, Jaera albifrons Leach and Asellus aquaticus L. (Crustacea: Isopoda). Acta Zool. (Stockh.) 59, 69-84 (1978).
- II. Nilsson, H. L.: Morphological variation of the compound eye of isopods - fine structural organization. MS 1982.
- III. Nilsson, H. L.: Fine structural changes caused by daylight exposure of the compound eye of Cirolana borealis (Crustacea). MS 1982.
- IV. Hallberg, E., Nilsson, H. L., Elofsson, R.: Classification of amphipod compound eyes - the fine structure of the ommatidial units (Crustacea, Amphipoda). Zoomorphologie 94, 279-306 (1980).
- V. Nilsson, D.-E., Nilsson, H. L.: A crustacean compound eye adapted for low light intensities (Isopoda). J. comp. Physiol. 143, 503-510 (1981).
- VI. Lindström, M., Nilsson, H. L.: The ERG spectral and absolute sensitivities of a deep-water crustacean (Cirolana borealis). MS 1982.
- VII. Nilsson, H. L., Lindström, M.: Retinal damage and sensitivity loss of a light-sensitive crustacean compound eye (Cirolana borealis): Electron microscopy and electrophysiology. MS 1982.

References to these papers will be made by using the roman numerals listed above.

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Introduction

One of the first accounts on the isopod eye is from Treviranus (1816), who noted that the terrestrial isopod Oniscus (one of the common woodlouses) "hat zwey Augen, die unter einem schwächern Vergrößerungsglas ein netzförmiges Ansehen haben"; i.e. the faceted pattern typical of compound eyes was disclosed. A contradictory result was noted by Müller (1829) in an amphipod. He observed that the cuticle overlying the eye of Gammarus was smooth and lacked facets on the surface. These differences in eye structure proved to be only the beginning of an ever increasing accumulation of data and facts on multifarious variation present in compound eyes.

Studies of thin sections with the light microscope extended the ken of knowledge to include the internal anatomy of the isopod and amphipod eyes. Initially, the major interest and debate were focused on whether the so-called rhabdom (rhabdites (Greek) = rod) was the light-receptive part or not of the eye and whether the insect and crustacean compound eyes were homologous structures.

With the advent of the electron microscope and its use in biology in the 1950's, the true nature of the rhabdom was revealed. It consists of microvilli from photoreceptor membranes (Goldsmith and Philpott 1957, Miller 1957, Wolken et al. 1957). At the same time, these membranes were shown to contain light-sensitive photopigments (Wald and Hubbard 1957).

The electron microscope made possible the investigation of ultrastructural and morphological adaptations present in the eyes of both insects and crustaceans. To date, the insect eye has been more extensively studied, both functionally and morphologically (Bernhard 1966, Bullock and Horridge 1965, Horridge 1975, Snyder and Menzel 1975). By comparison, only a few larger ultrastructural investigations of the crustacean eye have been published (Andersson 1979, Eguchi and Waterman 1966, Hallberg 1978, Odselius 1980). Thus, a basic knowledge of the ultrastructure of the crustacean eye is a desideratum and its absence is a serious drawback from both functional and phylogenetic points of view. However, salient details of the ultrastructure of the crustacean compound eye are slowly accumulating and the seven investigations summarized here are accounts on the fine structure of the eyes of two, hitherto neglected, large crustacean groups, the Isopoda and the Amphipoda (Crustacea: Peracarida).

The amount of morphological details presented in these investigations (papers I-VII) is considerable, and this might, on first sight, appear unimportant; but the observations are considered justifiable, because without a detailed knowledge of ultrastructure, a tenable approach to function and evolutionary speculation is very difficult.

In addition, a functional adaptation has been analysed in detail. The reason for making this special investigation was that forms living in moderate aquatic depths exhibit, in both isopods and amphipods, such characteristic features in eye structure that it can be expected there is a marked functional adaptation to low light intensities.

1. Comparative Morphology of the Isopod Eye (I, II, III)

The isopod eye is sessile (non-stalked) and is an apposition type*.

The number of ommatidia varies from a few to about 500-600. Sessile eyes are primarily present in isopods and amphipods.

Several morphological characters are shared by other peracarid crustaceans, and these are summarized in Table 1. The most prominent character is, perhaps, the consistency of the number of cells comprising the crystalline cones. Two cells form the cone and in addition two very small cone cells with distally situated nuclei are present. These do not, however, contribute to the cone formation.

Also the presence of distal and reflecting cells is a typical feature, although some species may lack them. Generally, the occurrence and distribution of the accessory pigment cells display great variation imposed by environmental demands (Hallberg 1978). Certain features are typical of the isopod eye, although they are not confined to them. This is particularly true of the biconvex lenses. Besides isopods these are present in crabs.

Only in a few species are the lenses replaced by an undifferentiated cuticle (see also amphipods, Chapter 3).

*Apposition eye = The crystalline cones and the rhabdoms are contiguous and the image falls on the proximal portion of the cone. This type is in contrast to the superposition eye = The crystalline cones and the rhabdoms are separated by a clear space and the first image is formed in the distal portion of the cone and some distance from the rhabdom. These images superimpose on the distal rhabdom tip. Several functional types are present (Kunze 1979, Land 1981).

Several morphological types of crystalline cones are present, but the most common one is spherical or pyriform in shape. Other types are interpreted as special adaptations to particular environmental demands. The general rhabdom type is the fused continuous one¹, which is often star-shaped in a cross section. A hitherto unique feature of the isopod eye is the presence of a so-called semi-fused rhabdom². A third rhabdom type is the fused layered rhabdom³, which only occurs in the valviferan isopods. This type of rhabdom has often been referred to as "the crustacean type", for it was first observed in the decapods ("typical crustaceans"). However, the occurrence of this rhabdom type is disjunctive and it is found in both crustaceans and insects.

The number of retinula cells varies between six and eight. Six cells are only found in valviferan isopods. These may also have a seventh cell, which contributes a rhabdomere distally. All other isopods possess seven or eight retinula cells. In those cases where eight cells are present, the additional cell contributes a distally situated rhabdomere similar to the distal cell in valviferans.

Two types of membranes that delimit the eye from surrounding tissues are present: viz. (1) a basement membrane and (2) a fenestrated

¹fused continuous rhabdom = the rhabdomeres are not optically isolated.

²semi-fused rhabdom = the distal part of the rhabdom, is as in 1, and the proximal part comprises optically isolated rhabdomeres.

³fused layered rhabdom = the orthogonally oriented rhabdomeres are stacked up on top of one another.

For rhabdom definitions, see also Elofsson (1976).

membrane together with an eye-capsule membrane. Both types are present in several isopod suborders.

Besides the general outline of the isopod eye described above, special morphological adaptations are seen in eyes adapted to the deep-sea environment (see Chapter 4).

In conclusion, the isopod eye exhibits a consistency as regards the number of cells comprising the dioptric elements, but other morphological features manifest greater variability.

2. Comparative Morphology of the Amphipod Eye (IV)

The compound eyes of amphipods are sessile and the number of ommatidia show great variation, ranging from a few to several thousand. The number and organization of cells forming the crystalline cones are identical to the isopods (Table 1). One exception to this common pattern is found in the ampeliscid amphipods, where all four cone cells are fully developed. However, their position on top of one another shows a resemblance to the distal position of the accessory cone cell nuclei, which favours an interpretation of a common pattern.

An almost consistent feature in all amphipods is the undifferentiated cornea (the ampeliscids possess a biconvex cornea). Unique is the number of retinula cells, which is five in all the species studied. No distally situated rhabdomere is present in amphipods. A fenestrated

membrane is usually found (except in ampeliscids). Disregarding the common features mentioned above, the amphipod compound eye can be classified into four sub-categories (types): (1) the gammarid type, which is a general type having no distinctive features; (2) the hyperid type, which possesses a large number of ommatidia and long crystalline cones (no pigment is present between the cones resulting in a typical transparent appearance of the eye); (3) the ampeliscid type, which has a tripartite aberrant lens eye, where a lens-like "vitreous body" is present between the cornea and the crystalline cones (this "lens" has been demonstrated to be part of the organ of Bellonci, a typical sensory organ in crustaceans (Elofsson et al. 1980)); and (4) the lysianassid type, which has morphological characteristics typical of eyes from deep-water living crustaceans, i.e. more or less a reduced dioptric apparatus, hypertrophied rhabdoms and a tapetal layer formed by reflecting pigment cells.

In conclusion, although a great variation is present in eye structure, certain features are characteristic of amphipod eyes as a whole, and other features make it justifiable to discriminate different eye subtypes.

3. Functional Adaptations to Low Light Intensities (III, V-VII)

The low number of photons available at moderate and great depths in the aquatic environment necessitates special adaptations of the eye if it is to function optimally in the prevailing light milieu. Such special adaptations are present in the eye of the

isopod Cirolana borealis, which is a deep-water, marine species. The adaptations studied were (1) morphological, (2) optical, (3) physiological (spectral and absolute sensitivities of the eye), and (4) special characteristics of the retinula cells and the photoreceptor membranes.

The morphological adaptations include large lenses, hypertrophied rhabdoms and an elaborate tapetal layer formed by reflecting pigment cells. Optically, the lenses impart to the ommatidia large acceptance angles, thus enabling the eye to collect photons from a large visual field. Further, a visual overlap is present between adjacent ommatidia. Together, these adaptations, then, satisfy the requirements for a high visual sensitivity. And this has been shown by electroretinogram measurements. Only a low number of photons were needed to evoke eye potentials. Moreover, spectral sensitivity measurements of the eye showed that a single photopigment system is present. Also the temporal resolution of the eye was low (a flicker fusion frequency of about 14 Hz).

Certain characteristics of the photoreceptor membranes were disclosed. These membranes are hypersensitive to strong light, which renders the eye useless (confirmed by sensitivity measurements). The membranes break down "irreversibly". A new type of photoreceptor membrane shortening was also disclosed.

4. Functional and Phylogenetic Aspects (I-VII)

Both isopod and amphipod eyes display a number of morphological adaptations, and these primarily involve the shapes of the dioptric elements and the rhabdoms. Unfortunately, little is known about the functional importance of several of these adaptations. A few examples will be mentioned directly below.

The biconvex cornea of isopods seems to be the main refractive part - lens - as is e.g. shown in Cirolana (V). In amphipods the crystalline cone constitutes the lens (Nilsson 1982).

Little is known of the function of the different rhabdom types. Most extensively studied are the fused layered and the fused continuous rhabdoms. The fused layered rhabdom is considered to be a special adaptation for perceiving polarized light (for review, see Waterman 1981), and this type is a good example of convergent development (Elofsson 1976). The fused continuous rhabdom, on the other hand, has been shown to increase absolute sensitivity (Snyder et al. 1973), and its presence in a number of isopod and amphipod eyes may reflect the particular circumstances (faint light) imposed by the light environment.

These few examples above of functional adaptations and the single example of convergence indicate the necessity of correlating morphology and function before phylogenetic speculations are bruited. The isopod and amphipod eyes engender problems as regards phylogenetic speculations since the eyes are readily modifiable

structures as regards environmental factors, such as varying conditions in their life habitats and particularly light regimes. And thus it can be concluded that the eye of isopods and amphipods can be validly employed in phylogeny, but it is imperative that great care be exacted and a large number of species be thoroughly studied.

CONCLUSIONS

1. The compound eyes of the isopod crustaceans demonstrate consistency as regards the number of cells comprising the dioptric apparatus. Receptor cells, which form the rhabdom, vary in number. Variation is also present with respect to the shapes of the crystalline cones and the rhabdoms.
2. Four types of amphipod compound eyes have been discriminated. One is a general type and the other three are morphologically well separated. The number of receptor cells comprising the rhabdom is constant in all four eye types.
3. The eye structure of deep-water forms are examples of convergent evolution. Morphology and function (optics, sensitivity of the eye, characteristics of the photoreceptor membranes) display definite adaptations to low light intensities. Excessive light causes retinal dystrophy and a loss of visual sensitivity.
4. It is concluded that the variability of the isopod and amphipod eyes is large within the common structural scheme included in the definition of the apposition eye. Phylogenetic speculations based on the eye structure require a detailed knowledge of form and function.

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	<u>ISOPODA</u>	<u>AMPHIPODA</u>	<u>OTHER PERACARIDA</u>
Corneagenous cells (no)	2 (0)	0	2 (0)
Biconvex lenses	yes	no*	no
Crystalline cone cells	2 + 2	2 + 2 (4)	2 + 2
Retinula cells	6 - 8	5	7 - 8
Rhabdom-types			
fused continuous	yes	yes	yes
fused layered	yes	no	yes
semi-fused	yes	no	no
Distal pigment cells	yes	yes	yes
Reflecting pigment cells	yes	yes	yes
Basement membrane	yes	no*	yes
Fenestrated membrane	yes	yes	no

* Except family Ampeliscidae

Table 1. Comparison between the number (no), morphological appearance and occurrence of different elements in the eyes of peracaridan crustaceans.

Morphological Variation of the Compound Eye of Isopods
(Crustacea) — Fine Structural Organization

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Running title: Fine Structure of Isopod Eyes

Key words: Eye — Crustacea — Anatomy

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Summary

1. The sessile isopod compound eyes are of the apposition type, and the eye compartments are delimited either by a fenestrated (FM) and an eye-capsule membrane or by a basement membrane (BM). Finally, the fine structure of the FM and BM is similar (except in Cyathura).
2. The number of cells constituting the dioptric elements are constant (except in Cyathura), viz. two corneagenous cells, two main crystalline cone cells as well as two accessory cells.
3. Morphologically, the crystalline cones vary. They are either vestigial, elongate or pyriform. A special type, present in Ligia, is bipartite.
4. The rhabdoms comprise three subtypes: (1) fused continuous, (2) fused layered and (3) semi-fused. Subtype 2 is present in suborder Valvifera. Other subtypes occur in more than one suborder.
5. The rhabdoms of shallow-water species have seven (suborder: Valvifera) or eight (suborders: Anthuridea, Flabellifera, Oniscoidea) retinula cells and possess a distal rhabdomere besides those of the main rhabdom. Deep-water valviferans possess six retinula cells, but no distal rhabdomere.
6. Two types of accessory pigment cells are present: distal pigment cells and reflecting pigment cells.
7. Light and darkness cause changes in eye structure and pigment migration in the retinula cells of shallow-water species (except in Cyathura,
8. Viewed morphologically, the dioptric elements and rhabdoms must be greatly affected by functional demands imposed by the environment. Hence, utmost circumspection must be taken as regards phylogenetic speculations based on eye structure.

Introduction

The diversity in the eye structure of isopod crustaceans, which was revealed by the light microscope about 100 years ago (Beddard 1888, Grenacher 1879, Hesse 1901, Parker 1891) has failed to be recognized in modern times. Only a few investigations dealing with the fine structure of the isopod eye have appeared (Edwards 1969, Nemanic 1975, Nilsson 1978, 1982). From both a functional (Lindström et al. 1982, Nilsson et al. 1981, Nilsson et al. 1982) and a phylogenetic (Nilsson et al. 1977) point of view, the isopod eye indicates interesting adaptations and especially so when taking into account the widespread ecological adaptations of these crustaceans. They are veritable inhabitants of the deep sea, as well as of the terrestrial environment.

Phylogenetic speculations based on the eye structure have been engaged in (Bullock et al. 1965, Dahl 1963, Elofsson 1976, Horridge 1977, Paulus 1979, Tiegs et al. 1958). However, the lack of extensive knowledge of the crustacean eye — perhaps with the exception of the decapods — has been an undue disadvantage in these speculations. The present results from the comparative analysis of the fine structure of the isopod eye add new basic facts disclosing the diversity in fine structure of the isopod eye .

Material and Methods

The heads of the specimen were prefixed according to Karnovsky (1965) (Sphaeroma also in 2.5% GA in phosphate buffer, pH 7.2) in 0.2 M sodium cacodylate buffer (pH 7.2) for 4 hours at 4°C.

Postfixation was performed in 1-2% OsO₄ for 1-2 hours at 4°C. The tissue was dehydrated in an alcohol series, blockstained in 0.5% uranyl acetate/1% phosphotungstic acid (in absolute alcohol) and embedded in Vestopal W. Sections on grids were stained in 1% uranyl acetate (aq. dest.) for 20 min and in alkaline (NaOH) 0.5% lead citrate (aq. dest.) for 1-2 min, and examined in a Zeiss EM 10 and in a Jeol 100 CX electron microscope. For light microscopy, 1-2 µm sections were cut and stained according to Richardson et al. (1960). Eyes were dark-adapted (except Idothea emarginata and I. neglecta) for 12 hours and dissection and fixation were carried out in red safety light (except Mesidothea, which was dissected in white light because of dissection difficulties). Light adaptation was accomplished by light from a 60 W bulb for 1-2 hours (distance 0.6 m) or by exposure to daylight for about 1 hour.

Studies were made on central ommatidia of the marine and brackish water species listed below (Table 1). They were obtained on the west and southwest coast of Sweden, except Mesidothea, which was collected in the Tvärminne Storfjärd, Tvärminne, Finland.

Table 1. Species upon which fine structural investigations of the eye were performed. Depth ranges, where the animals usually are trapped, are given in metres according to Dahl et al. 1965, 1966, Naylor 1972, Stephensen 1948. (Values within brackets are the deepest recorded locality.)

<u>Suborder</u>	<u>Species</u>	<u>Depth range (m)</u>
Anthuridea	<u>Cyathura carinata</u> , Kröyer 1848	0-6
Flabellifera	<u>Sphaeroma hookeri</u> , Leach 1814	0-a few metres
Valvifera	<u>Astacilla longicornis</u> , Sowerby 1806	18-54 (752)
	<u>Idothea baltica</u> , Pallas 1772	0-20' (340)
	<u>Idothea emarginata</u> , J.C. Fabricius 1793	6-20 (40)
	<u>Idothea neglecta</u> , G.O. Sars 1899	10-40
	<u>Idothea viridis</u> , G.O. Sars 1899	0-6 (30)
	<u>Mesidothea entomon</u> , Richardson 1905	5-225
Oniscoidea	<u>Ligia oceanica</u> , L. 1767	amphibian

Results

Some general features of the investigated isopod eyes are summarized below. Exceptions are indicated and described more thoroughly in subsequent sections. These later sections also contain a more detailed description of the rhabdom and crystalline cones, which vary most in the isopod compound eyes.

Further adaptations, where they occur, to darkness and light are described. The eyes of Ligia oceanica (Edwards 1969) have previously been studied and only new facts are reported here. Common features for the investigated isopod eyes are the following: They are sessile and of the apposition type (definition according to Exner 1891) and are situated on the lateral margins of the head segment. The ommatidia point in many directions, predominantly laterally and dorsally (Figs. 1, 2a, 3, 4, 5, 6).

The packing of the ommatidia is hexagonal and the individual facet is either round or hexagonal (see also Cyathura). The number of ommatidia varies as regards the species (values reported below in the text refer to adult specimens).

Three types of ommatidial-delimiting membranes in the isopod eye can be discriminated: (1) a basement membrane, (2) a fenestrated membrane and (3) an eye-capsule membrane. The basement membrane (1) delimits a space where all structural parts of the eye are contained (Fig. 7a) and this membrane is found in Cyathura, Astacilla and Ligia.

The fenestrated membrane (2) divides the eye into a distal portion -- which houses the dioptric elements, the accessory pigment cells and the rhabdoms -- and a proximal portion, which contains the nuclear portion of the retinula cells and glial cells (Fig. 7b). This membrane type is found in Sphaeroma, Idothea and Mesidothea.

Finally, the eye capsule membrane (3) delimits the nuclear region of the retinula cells in those eyes that possess a fenestrated membrane (Fig. 7b).

The fine structure of the basement and fenestrated membranes is similar. These membranes are formed distally by cellular sheets originating from expanded end-feet of distal pigment cell extensions. Frequently, fibrillar electron-dark densities are contained in the end-feet and in Astacilla these are prominent (Fig. 28). Proximally, a glial cell sheet is present. Each sheet is covered by a basal lamina, and between the two laminae an extracellular electron-dense substance of varied thickness is present (Fig. 9). The thickness of the two membranes varies between 1-6 μm .

The accessory cone cell processes (see below) are joined to the fenestrated membrane, and their terminals are usually ensheathed by the extracellular material (Fig. 10). At regular intervals, the retinula cell dendrites and axons pass through openings in the membrane.

The fine structure of the eye-capsule membrane is simple; a glial cell sheet is covered by a basal lamina (Fig. 36) and the thickness is usually about 0.2-0.3 μm .

The corneal lenses are secreted by two corneagenous cells. These cells project down into the eye for some distance and envelop the crystalline cones (Fig. 8) (except in Cyathura). The crystalline cones are formed by two principal cone cells and, in addition, two accessory cone cells are located at the ends of the suture line between the two principal cone cells (Fig. 8) (except in Cyathura). All nuclei are located distally. The fine structure of the cone appears as an electron-dense amorphous core, which is surrounded by a thin granular zone, and outside this, the cone cell cytoplasm is present (Fig. 8, inset) (see also Ligia). Microtubule-filled processes from the accessory cone cells extend to the basement (or fenestrated) membrane, and the processes are always found close to, and on opposite sides of, the rhabdom (Figs. 20, 21, 27, 30, 34). Occasionally, extensions from the corneagenous cells are present outside the retinula cells, but it has not been ascertained whether or not they continue to the fenestrated membrane.

The fused rhabdoms are either layered or continuous (except in Ligia, which has separate rhabdomeres proximally) and are formed by 6 to 8 retinula cells. Axons from one ommatidium are ensheathed by glia (Fig. 24). The retinula cells contain pigment granules that have the ultrastructural characteristics of ommochromes

(Elofsson et al. 1973), and with few exceptions the diameters of the granules lie between 0.4 and 0.8 μm . Below the basement (or fenestrated) membrane, the retinula cells are ensheathed by glial cells (Figs. 23, 24, 39).

Accessory pigment cells are present as distal and reflecting pigment cells (Fig. 8) (except in Cyathura). Six distal pigment cells surround each ommatidium. (Thus, each cell is shared by at least three ommatidia, when the packing is hexagonal.) The nuclei are distal in position and the cells contain numerous electron-dense pigment granules with a fine structure similar to those of the retinula cells (Fig. 8).

Proximal to the crystalline cone the distal pigment cells continue as slender processes or thin sheets to the basement (or fenestrated) membrane where they terminate as widened end-feet (Figs. 9, 22, 28). The number and expansion of the reflecting pigment cells vary interspecifically, but usually they extend from the cornea to the fenestrated or basement membrane (see also Sphaeroma and Astacilla). The fine structural appearance of the membrane-bound granules varies and is inconstant intraspecifically. Either the granules appear "empty" or an electron-dense material is left or a crystalline-like residue is present (Figs. 21, 23, 34, 43). The size of the granules ranges between 0.3 and 0.5 μm in diameter.

Suborder: Anthuridea

Cyathura carinata (Fig. 11)

The small oval-shaped compound eyes ($60 \times 40 \mu\text{m}$) appear externally as two reddish-brown pigmented spots.

The eye is bent around the lateral side of an extension of the head (Fig. 1), which imparts to the ommatidia a dorsal, lateral, and ventral orientation (see below). No outer facet outline is observed. The cuticle forms a wide transparent zone, c. $60 \mu\text{m}$ in diameter, around the eye. Outside of this, the cuticle is pigmented. Beneath the transparent cuticle, the eye is contained in a cup (Fig. 11), which is delimited towards the surrounding tissues and haemocoel by a basement membrane constituted of peripherally located glial cells and a basal lamina (Fig. 12). The eye contains seven ommatidia. Three of these are oriented obliquely dorsolaterally, two are oriented laterally and the remaining two obliquely ventrolaterally.

Dioptric Elements

The eye is covered by a $20 \mu\text{m}$ thick undifferentiated cuticle (Fig. 11). It is formed by a single layer of interdigitating hypodermal cells (c. $10 \mu\text{m}$ thick). The vestigial spherical crystalline cones are small, less than $8 \mu\text{m}$ in diameter. Frequently, the cone is bipartite and the two cone cores belong to either one or two cells (Figs. 13, 14). When one cone is present, it is formed by a single cone cell.

Two cells with cytological characteristics similar to the hypodermis cells are located on opposite sides of the cone(s). Their position is similar to the position of the accessory cone cells (Fig. 13). They can be interpreted as homologues to corneogenous cells owing to their cytological characteristics and as accessory cone cells owing to their position.

Retina

The retina contains only two cell types: glial cells and retinula cells. Thin sheets from the glial cells encapsulate the retinula cell-axons in bundles of eight.

The retinula cells form a fused continuous rhabdom, which comprises eight rhabdomeres distally. The remainder of the rhabdom is formed by seven rhabdomeres. Both the width and the length are c. 22 μm , making the rhabdom a compact structure. Most distally, the rhabdom is fairly round in a cross section and the eighth rhabdomere is situated centrally. Microvilli protrude from a club-like extension of the eighth retinula cell (Fig. 15). The cytoplasm contains few electron-dense pigment granules.

The main rhabdomere is star-shaped in a cross section (Fig. 16) and microvilli originate from wedge-shaped folds of retinula cells. The microvillar tips from opposing rhabdomeres are separated by a thin (40-50 nm-wide) cytoplasmic sheet originating from one of the retinula cells (Fig. 17).

The retinula cells are heavily pigmented by two populations of membrane-bound, electron-dense pigment granules, diam. c. $0.5\ \mu\text{m}$ and $0.7\ \mu\text{m}$, respectively. The larger granules are few and are primarily located in the retinula cell proximal to the rhabdom.

Flabellifera

Sphaeroma hookeri (Fig. 19A)

The dome-shaped, black pigmented, eyes comprise about 100 ommatidia each (Fig. 2c). A row of setae surround the perimeter of the eye (Fig. 2c). Microvilli generally occur between the lenses (Fig. 2b).

Dioptric Elements

The cuticular lenses (diam. $25\ \mu\text{m}$) are biconvex and the strongest curvature is towards the interior of the eye.

The crystalline cones are pyriform in shape and the diam. of the widest part and the length are roughly equal, about $28\ \mu\text{m}$.

Retinula Cells and Rhabdom

Seven retinula cells (R1-7) comprise the main portion of the fuse continuous rhabdom and an eighth receptor cell (R8) contributes with a rhabdomere distally (the latter comprises about 80% of the

rhabdom area and has a depth of about 2 μm) (Fig. 18). Retinula cell eight is located on both long sides of the rectangular rhabdom (when seen in a cross section) and the microvilli project from opposing sides.

The cytoplasmic portion of R8, which is located between R3 and R4, may appear as an isolated cell island, but it is continuous with the main portion of the cell in the distal-most part of the rhabdom (Fig. 19B). The microvilli of R8 have thicknesses varying between 150 and 300 nm and the electron-lucent cytoplasm is characterized by few electron-dense pigment granules (diam. about 0.6-0.8 μm) (Fig. 18).

The dendrite of R8 runs outside R7 and the axon is considerably thinner than those of R1-7 (Fig. 23).

The main portion of the rhabdom is formed by four equally large cells (R1, 2, 5, 6) and by three small cells (R3, 4, 7) (Figs. 20, 21). Two of the small cells, R3, 4, are located opposite the third small one, R7 (Fig. 20), and their microvilli (diam. about 100 nm) are parallel. They meet end-to-end in the centre of the rhabdom (Fig. 20, inset).

The microvilli of R1 and R2, on the one hand, and R5 and R6 on the other, are oriented towards each other at angles of 60-90°. The microvilli are about 50 nm thick. No palisade is present (Fig. 20).

The dendrites from one ommatidium penetrate the fenestrated membrane in a bundle of eight, and both the individual dendrites and the

bundle are encapsulated by glial cells. The retinula cell dendrites corresponding to the four large, three small and the eighth retinula cell can be identified within the dendrite bundle (Fig. 23).

Accessory Pigment Cells

The distal pigment cells contain small granules (about 0.1-0.2 μm in diam.) close to the crystalline cone and large granules (diam. c. 0.3-0.6 μm) are distributed throughout the cytoplasm (Fig. 8).

The proximal extensions of the distal pigment cells are extensive and more or less ensheath each ommatidium and only a small number of pigment granules are contained in the cytoplasmic sheets. One population of reflecting pigment cells extends from the cornea to the soma region of the retinula cells (below the fenestrated membrane). The reflecting pigment cells project in between and fill the space between the retinula cells (Figs. 20, 21). In addition, another population of reflecting pigment cells is found below the fenestrated membrane. Cytoplasmic sheets and extensions from these cells intermingle between the retinula cell soma (Fig. 22) and distal to the eye-capsule membrane they widen into cellular sheets which surround the eye.

Changes in Light and Darkness

Movements of the eye-shielding pigments are evoked by changes in light regime. In light, the granules are located close to the rhabdom (Figs. 18, 20) and the concentration is highest around the

distal rhabdom tip. In darkness, the granules migrate proximally and leave a pigment-free zone around the rhabdom. The granules are found in abundance proximal to the fenestrated membrane. In addition, the volume of the retinula cells decreases in the rhabdom region and the reflecting pigment cell volume increases. Hence, the rhabdom is surrounded by dilated reflecting pigment cells (Fig. 21).

No pronounced change in rhabdom volume is found. The shape of the crystalline cone changes from pyriform in light to spherical in darkness. The end-feet of the distal pigment cells are broader in darkness and contain a number of irregularly shaped vesicles. Some are lysosome-like in appearance and contain what resemble pigment granules (Fig. 22).

Valvifera

Astacilla longicornis (Fig. 19C)

The dome-shaped compound eyes of Astacilla protrude from the body more than in other isopod eyes (Fig. 3).

The eye comprises about 350-450 ommatidia and the packing is hexagonal (Fig. 3). The transparent eyes have a pale-golden yellow hue and a small, distinct and black pseudopupil is present both in light and darkness.

Dioptric Elements

The corneal lenses are hexagonal in outline and slightly biconvex (the central thickness is about 30 μm).

The crystalline cones are elongated, about 130 μm long, and the distal diameters are c. 60 μm while proximally the cones narrow to diameters of about 8 μm (Fig. 25).

Retinula Cells and Rhabdom

Six symmetrically arranged and equally large retinula cells form a fused layered rhabdom, which has a wide extracellular palisade (Fig. 27). Further details on the fine structure of the rhabdom are presented in Nilsson et al. (1977). The retinula cell nuclei are located distally at the proximal level of the cone (Figs. 25, 26) and the cells continue as slender, non-pigmented processes (Fig. 26) to the rhabdom tip, where they expand and form the rhabdomeres (rarely the retinula cell nuclei may be observed in the rhabdom region).

Small electron-dense granules (diam. about 0.15-0.20 μm) are located close to the palisade, and larger granules (diam. about 0.4 μm) are located outside the small ones (Fig. 27).

Accessory Pigment Cells

The nuclei of the distal pigment cells are located about halfway up the crystalline cones (Fig. 25), and the cytoplasm in this region is confined to thin processes that extend through the reflecting pigment cell layer (see below). Occasionally, a few pigment granules are found in this distal region, but the highest concentration is in the region where the crystalline cone and the rhabdom meet (Figs. 25, 26) and distal to the basement membrane (see below). Around the distal rhabdom tip, the distal pigment cells expand and form a heavy pigmented layer, which is about 20 μm thick and where the granules are about 0.6-0.8 μm in diameter.

Proximal to the rhabdom tip the distal pigment cells extend as thin sheets between the ommatidia, and they once again dilate and terminate as a 10-15 μm thick layer above the basal lamina (Fig. 28). The pigment granules are abundant in this region and the diameters of the granules are larger, c. 0.8-0.9 μm .

Reflecting pigment cells are distal in position and occupy the space between the corneal lenses and the distal pigment cell layer around the rhabdom tip (Figs. 25, 26).

Changes in Light and Darkness

No migration of the eye pigments was found. A slight increase (20-40%) of the distal rhabdom area is caused by darkness and the diameter of the crystalline cone adjusts accordingly.

Idothea baltica (Fig. 19D), I. emarginata, I. neglecta, I. viridis

The eyes are dome-shaped, black-pigmented and situated laterally on the head segment (Fig. 4). The round lenses are hexagonally packed.

The size proportions of the elements of the eye vary in the different Idothea species (Table 2) and the fine structure, apart from the rhabdom, is similar.

Dioptric Elements

The round corneal lenses are biconvex and exhibit the strongest curvature on the internal surface. The shape of the crystalline cone is pyriform.

Retinula Cells and Rhabdom

The number of retinula cells that comprise the rhabdom and the different types of rhabdom that occur in the Idothea species are summarized in Table 2.

Idothea baltica, I. emarginata and I. viridis possess seven retinula cells (R1-7) (see also I. neglecta). The seventh retinula cell (R7) contributes distally with a rhabdomere (Fig. 29) and comprises about 80-90% of the central rhabdom area. The depth of the rhabdomere is about 2 μm .

Table 2. Rough estimates of some size dimensions (in μm) of the eye in Idothea species (values valid for adult animals and light-adapted eyes). Included are also some characteristics of the rhabdom. +1 = retinula cell with a distally located rhabdomere.

	<u>I. baltica</u>	<u>I. emarginata</u>	<u>I. viridis</u>	<u>I. neglecta</u>
<u>Number of ommatidia</u>	120	200	100	200
<u>Corneal lens</u>				
diameter	35	55	30	45
thickness (centrally)	18	30	12	25
<u>Crystalline cone</u>				
diameter	30	40	20	35
length	40	50	30	50
<u>Rhabdom</u>				
number of retinula cells	6+1	6+1	6+1	6
type	layered	layered	layered	continuous
cross-sectional appearance	squarish-rectangular	squarish-rectangular	oval	star-shaped
width	5	7	5	5-10
length	65	75	40	75

Microvilli originate at right angles and orthogonal to the light from a club-like extension of R7, which protrudes into the centre of the rhabdom. A few electron-dense pigment granules (diam. about 0.5-0.7 μm) are present in the cytoplasm outside the rhabdom (Fig. 29). The axon runs outside R1 and R6 (Fig. 30).

The main rhabdom is formed by four large rhabdomeres (R2, 3, 5, 6) orthogonally oriented to two small rhabdomeres (R1, R4) (Fig. 30). In a longitudinal section, the rhabdom appears layered or serrate (Fig. 32).

Because the cross-sectional area of the two orthogonal microvillar layers differs, the rhabdom appears alternately square-like and rectangular. This causes the rhabdom to appear wavy in a longitudinal section, and this is most pronounced in I. emarginata (Fig. 33). The microvilli are 60-70 nm wide in all species of Idothea. A small extracellular palisade is present.

The rhabdom of Idothea neglecta is fused continuously and is formed by six reticular cells. In cross section, the rhabdom is round in its most distal position; but otherwise, it is star-shaped (Fig. 34).

A tendency towards a lost layered appearance can be conceived because the microvilli of a rhabdomere are shorter at regular intervals (wavy appearance in a longitudinal section), although the toothed appearance is never seen. Further, the microvilli of R1 and R4 are aligned towards each other, and are partly orthogonal to R2, 3, 5, 6. A small extracellular palisade is present.

Finally, the retinula cells in I. neglecta contain a number of large (diam. c. 2 μ m) granules, that are not found in the other Idothea species (Fig. 35).

Changes in Light and Darkness

Two species, I. baltica and I. viridis, were light- and dark-adapted and the results were similar.

Pigment migration occurs in the retinula cells, and when the eye is exposed to light the pigment concentrates both around and close to the rhabdom. The highest concentration of pigment is found about the distal rhabdom tip. In darkness, the pigment disappears in the rhabdom region (only a few granules are found peripherally in the retinula cells). The granules are copious proximal to the fenestrated membrane. In addition, the volume of the retinula cells radically decreases in the rhabdom region and this seems to be compensated for by an increase in volume of the reflecting pigment cells. The net result is that only thin unpigmented sheets of retinula cell cytoplasm remain around the rhabdom, and instead an extensively developed reflecting pigment cell layer surrounds the rhabdom (Fig. 31). No migration of pigment is found in the distal pigment cells.

The cross-sectional area of the rhabdom increases by more than 100% and the proximal cone tip adjusts in diameter accordingly.

Mesidothea entomon (Fig. 19E)

The small dark-pigmented eyes comprise about 40 ommatidia each and are situated on the dorsal side of the head segment and are directed upwards (Fig. 5).

Dioptric Elements

The round corneal lenses are biconvex (about 40 μm in thickness) and the pyriform crystalline cones are c. 40 μm in diameter and c. 50 μm in length.

Retinula Cells and Rhabdom

Seven retinula cells form a fused continuous rhabdom, which is round in a cross section immediately below the cone (diam. c. 18 μm) but otherwise star-shaped (diagonal, c. 30 μm) (Fig. 37). The star-shaped appearance of the rhabdom is engendered by microvilli-coated folds arising from the retinula cells, which in turn are concentrically arranged. Microvilli originate at right angles to the cell surface. Distally, the rhabdomeres are equally large, but below this level the seventh rhabdomere is smaller (Fig. 38). The length of the rhabdom is c. 65 μm . No palisade is present and the microvilli are 60-100 nm wide.

The electron-dense pigment granules of the retinula cell are moderate in number, and only around the distal rhabdom tip and immediately above the fenestrated membrane is a higher concentration present.

Thin glial cell sheets (nuclei in the rhabdom region) separate the retinula cells.

In some eyes another cell type is present among the soma of the retinula cells. It is characterized by stacks of continuous membranes that fill the major portion of the cell. The membranes are mostly parallel, although local swellings and whorl formations are common (Fig. 39). The nucleus is located at one end of the cell. A number of electron-dense pigment granules are present (about 0.6-0.8 μm in diameter). Lysosome-like bodies and "empty" vesicles are frequent. All cells are ensheathed by glia (Fig. 39). It has not been ascertained whether these cells are constant features of the eye.

No changes were found in light and in darkness.

Oniscoidea

Ligia oceanica (Fig. 19F)

A re-investigation of the eye of Ligia showed that the following morphological structures differed from those described previously (Edwards 1969, Hewitt 1907, Ruck et al. 1954): (1) the dioptric elements, (2) the rhabdom, (3) the accessory pigment cells. Only new results are presented.

Dioptric Elements

The cornea is slightly biconvex and the crystalline cone is elongated. The latter conforms to the general description of the isopod cone with regard to the number of cells.

Structurally, the cone is divided into a distal and proximal portion (noted by Hewitt 1907). The distal part (diam. c. 55 μm) is similar to the cones of other isopods and consists of an amorphous electron-dense core that is surrounded by cytoplasm. The proximal part is a rod-like extension of the crystalline cone cell cytoplasm (Figs. 40, 41) (width c. 40 μm , length c. 50 μm) and it abuts the distal rhabdom tip (Fig. 41). Both the distal and the proximal portions of the cone are surrounded by the corneagenous cells.

Retinula Cells and Rhabdom

An eighth receptor cell (R8) is present on top of the rhabdom and it bears a rhabdomere, which lies in a depression between the proximal crystalline cone tip and the distal rhabdom tip (Fig. 41). The shape of the rhabdomere is "fist-like" (thickness c. 5 μm) and the microvilli emerge from a club-like extension of the retinula cell (the arrangement is similar to that described in Idothea, except that the central cytoplasmic portion is absent).

The microvilli are oriented parallel to those of R1-7 of the main rhabdom and the axon of R8 is located between R1 and R7 (Fig. 19G).

The nucleus is located at the level of the rhabdomere. (A nucleus in this position was previously reported by Ruck et al. (1954), but no rhabdomere was observed.) The cytoplasm of the soma contains electron-dense pigment granules (diam. c. 0.6-0.7 μm) that are similar in fine structure and size to those of the other retinula cells. The microvilli are 50-60 nm wide.

Accessory Pigment Cells

One reflecting pigment cell is present in each ommatidium. The nucleus is basal in position and is located between the open rhabdomeres of the proximal rhabdom. The cytoplasm contains reflecting pigment granules as judged by their fine structure. The granules appear either "empty" or a crystalline residue is present within the bounding membrane (Fig. 43).

Changes in Light and Darkness

No changes were discriminated in the eye's fine structure. However, by studying the eye in situ employing light microscopy, changes can be visualized. The dark-adapted eye is black in appearance, but after illuminating the eye for more than 1-2 min, golden yellow streaks appear between the ommatidia.

Discussion

Morphological Aspects

Eye

The gross structure of the isopod eye can be classified into two general types with respect to the organization of their delimiting membranes. One type, which includes Sphaeroma (Flabellifera), Idothea and Mesidothea (Valvifera) possesses a fenestrated membrane and the second type, including Cyathura (Anthuridea), Astacilla (Valvifera) and Ligia (Oniscoidea) possesses a basement membrane.

A fenestrated membrane is also found in Idothea metallica (Peabody 1939), in Jaera (Asellota) (Nilsson 1978) and a homologous membrane, although located proximal to the retinula cell nuclei, is present in Cirolana (Flabellifera) (Nilsson 1982b). Asellus (Asellota) (Nilsson 1978), on the other hand, possesses a basement membrane, by definition, although it was originally designated fenestrated. Isopods belonging to the Oniscoidea have basement membranes. Thus, it appears that membrane organization is not restricted to specific taxa because these membrane types occur independently in different suborders of the Isopoda.

The fine structure, however, of the fenestrated and the basement membranes is similar in almost all species (see below) and this accords with earlier investigations (Nilsson 1978, Nilsson 1982b).

The exceptions constitute only minor differences and they are present in Cyathura (this paper) and Porcellio (Nemanic 1975). The fenestrated membrane of amphipods differs owing to the lack of the distal pigment cell sheet (Hallberg et al. 1980); but disregarding this, the general organization outlined above seems to be in agreement with that of all peracaridan crustaceans (Odselius et al. 1981).

Dioptric Elements

The cuticular lenses are biconvex and formed by two corneagenous cells. The crystalline cones are formed by two principal cone cells, and two accessory cone cells are present. The consistent presence of two accessory cone cells is in agreement with other isopods investigated (Nilsson 1978, Nilsson 1982b). Accessory cone cells are also found in amphipods (Hallberg et al. 1980), tanaids (Andersson et al. 1978) and mysids (Hallberg 1978). Based on the cited works and the present investigation, accessory cone cells are now a consistent element of several peracaridan suborders. Only in Cyathura (Asellota) is this regular pattern changed (see below).

Four morphological subtypes of crystalline cones can be discriminated (Fig. 44): (1) vestigial cones in Cyathura (Anthuridea); (2) pyriform cones in Sphaeroma (Flabellifera), Idothea and Mesidothea (Valvifera); (3) elongated cones in Astacilla (Valvifera); and (4) bipartite cones in Ligia (Oniscoidea).

The first type, i.e. vestigial cones, is found in a number of isopods with vestigial or degenerated eyes (Eggert 1927, Kosswig et al. 1936,

de Lattin 1939). The second type is present in shallow-water asellotes (Nilsson 1978), in flabelliferans (Beddard 1888, Nilsson 1982b) and in oniscoids (Debaisieux 1944, Nemanic 1975). The crystalline cones of Astacilla are distinctly elongated and terminate as thin necks on the rhabdom tips (third type). The morphological appearance displays interesting similarities to the cones of the pelagic amphipods (Ball 1977, Hallberg et al. 1980; Meyer-Rochow 1978). This is further emphasized by the transparency of the region occupied by the dioptric elements, which is seen by inspection of live animals.

Ligia, on the other hand, possesses a crystalline cone (fourth type) that resembles the cone of the branchiopod Artemia salina, i.e. when considering the division of the cone into a distal and proximal part (Elofsson et al. 1975, Nilsson et al. 1981b). The distal part of the cone, however, differs in appearance in the two species: in Artemia it is electron-lucent (containing glycogen); in Ligia electron-dense. The proximal part consists of an elongation of the crystalline cone cell cytoplasm, and this is rather similar in both species.

Rhabdom (Fig. 44)

Three major rhabdom types were found in this investigation: (1) fused continuous, (2) fused layered, and (3) semi-fused. A fourth type, present in Porcellio (Oniscoidea) is characterized by an eccentric cell (Nemanic 1975, Nilsson unpublished).

(For rhabdom definitions, see Elofsson 1976.) The fused continuous rhabdom (type 1) has microvilli that are continuous along the entire length of the rhabdomere. This type is found in Cyathura, Sphaeroma and Mesidothea. It is also present in some other isopods that belong to the suborders Asellota (Nilsson 1978) and Oniscoidea (Debaisieux 1944, Nemanic 1975). Often the cross-sectional appearance of this rhabdom type is star-shaped.

The fused layered rhabdom (type 2) possesses toothed rhabdomeres, i.e. the microvilli project in layers at regular intervals and they are separated by smooth membrane portions. These are occupied by orthogonally oriented microvilli from adjacent retinula cells. This organization is typical of the valviferan isopods Astacilla (see also Nilsson et al. 1977), and Idothea and it seems to be exclusively found in this suborder. The rhabdom of I. neglecta possesses what seems to be a type 1 rhabdom. However, the aberrant morphology of the rhabdomeres, characterized by four reduced and two normal rhabdomeres, may obscure a layering. The other exception is Mesidothea, which has a type 1 rhabdom (see above).

The third rhabdom type is present in Ligia and has been designated semi-fused (def. Nilsson et al. 1981a). It is characterized by distally fused and proximally separated rhabdomeres. The space between the separated rhabdomeres is occupied by a basal reflecting pigment cell. This cell was interpreted as an eighth receptor cell, although the pigment cell character was noted (Edwards 1969). The semi-fused rhabdom is, besides the one exception in Ligia, found in

the flabelliferan isopods, where it has been described in Cirolana (Beddard 1888, Nilsson 1982b) and Aega (Beddard 1888). It is also possibly present in Arcturus (Valvifera, Beddard 1890). However, the fine structural details of the rhabdom of Cirolana (Nils. n 1982b) differ from those in Ligia (Edwards 1969).

The number of receptor cells comprising the rhabdom is six, seven or eight. All shallow-water species of Idothea have seven retinula cells. This number was also noted by Peabody (1939) in I. baltica and I. metallica. The seventh cell, when present, contributes a rhabdomere distally. Idothea neglecta and Astacilla, which live in somewhat deeper waters, lack the seventh cell. The deep-water-living Mesidothea is an exception, having continuous rhabdomeres in all the seventh cells, although the seventh rhabdomere is larger distally and smaller proximally. The other shallow-water species, including Ligia, possess eight retinula cells, and the eighth cell contributes a rhabdomere distally. Only in the terrestrial isopod, Oniscus, is the number of cells different, as the rhabdom of this species comprises 14-17 retinula cells (Debaisieux 1944). Further discussions on the occurrence of distal receptor cells in crustaceans are found in Hallberg (1977), Nilsson (1978), Waterman (1981).

Functional and Phylogenetical Aspects

"Morphological evolution cannot be understood without an adequate knowledge of function" (Manton 1977). This statement is certainly relevant for the isopod compound eye. The varied appearance in fine structural details of the various components of the eye within the different suborders of the Isopoda makes it difficult to establish a common unit structure for the eye — if present at all. With this in mind, some functional aspects of the isopod eye in relation to the life strategies of the animals will be discussed first. After this, the morphological features of the eyes will be discussed from a phylogenetic point of view.

Functional Aspects

Cyathura is a predator (Johnson, personal communication) and lives in tubes in shallow water (Dahl et al. 1965). The vestigial crystalline cones and the undifferentiated cuticle of the eye can be attributed to the tube-dwelling life. Because of this adaptation, Cyathura has been able to dispense with the facets and instead of these has obtained a smooth outer cuticle that possibly facilitates the cleansing of the eyes. Further, the diverging ommatidial directions should make detection of motion possible despite the few number of ommatidia. This has also been verified, for the animals suddenly withdraw into the tubes if a hand is moved above them (Johnson, personal communication). The well-organized receptor elements also suggest a visual function. But compared with other

isopod eyes, the eye of Cyathura is considered to be specialized by reduction, and this is also proven by the absence of the distal pigment cells.

Astacilla has comparably large eyes (measured with an isopod standard) and well-developed crystalline cones similar in appearance to those of pelagic amphipods.

A function of the cones in these amphipods has been proposed by Land (1981) and Nilsson (1982a). According to these investigators, the special refractive properties of the cones and the transparency of the eyes are imposed by the necessity to see in dim light and to avoid detection by predators. A similar function would seem applicable to the eye of Astacilla, which seems reasonable on morphological grounds. However, pertinent facts on the importance of vision in Astacilla species lack. Only limited information on their biology is available. Astacilla longicornis lives on bryozoans and hydroids (Dahl et al. 1965), and this species seems to be a filter feeder with a capacity to detect small objects (Attramadal et al. unpublished). These facts were also verified by Schieke (1973) on an undetermined species of Astacilla.

The rhabdoms of Astacilla and Idothea (except I. neglecta) are layered. This rhabdom type has been shown, functionally and behaviourally, to be specifically adapted to perceive polarized light (for a review, see Waterman 1981), and this has also been

concluded by theoretical calculations (Snyder et al. 1973). Nothing is known, however, of the significance of this rhabdom type in regard to the biology of valviferan isopods.

The presence of an additional receptor cell on top of the rhabdom in shallow-water forms is a consistent feature of all the suborders. The function of the distally situated receptor cell in malacostracan crustaceans is unknown. However, recent investigations on the so-called eighth retinula cell in the rhabdom of the crayfish Procambarus have shown that it is a violet-sensitive receptor (Cummins et al. 1981). This cell has obviously evolved, as it is usually absent in deep-water-living forms, to meet the demands of the light milieu of shallow waters or on land.

At present, nothing can be stated about the functional significance of the dioptric apparatus of Ligia in relation to the life habits of this species. The semi-fused rhabdom is another enigma. Based on anatomical and optical considerations of a similar type of rhabdom in Cirolana, a hypothesis has been advanced (Nilsson et al. 1981a). It proposes that the separate rhabdomeres function as individual channels in bright light and in darkness all signals from the receptors are neurally pooled.

Simple light and dark adaptational experiments were performed. The results revealed that in the shallow-water species, except Cyathura and Ligia (undetermined changes in the eye pigment colour was, however, observed in Ligia by "pupil inspection"), pigment migration occurs in the retinula cells. Further, both the retinula cells and

the reflecting pigment cells change their volume (shape) in the rhabdom region. The cross-sectional area of the rhabdom increases. These changes can be correlated with the mode of life, since the deep-water-living species lack these adaptations. But the light and dark adaptations do not severely alter the morphology of the eye, and they cannot be used in phylogenetic speculations. These must subsequently be looked for in the morphological construction of the eye in correlation with the life strategy of the animal.

Phylogenetical Aspects

Few structures of the eye seem to be shared by members of the different suborders of Isopoda, but those most in common are as follows: sessile eyes that are of the apposition type; the biconvex lenses are formed by 2 cells; the crystalline cones are formed by 2 cone cells, and in addition two accessory cone cells are present; and finally, the fine structure of the basement and fenestrated membranes is similar.

The rhabdom shows a number of features that can be interpreted as convergent evolution (convergence is here defined as structural details of the eye from different ancestries evolve to subsequently resemble each other. No distinction is made between convergent and parallel evolution). Two examples will be mentioned and the first is the layered rhabdom of valviferan isopods. This rhabdom type is also found in decapods (Rutherford et al. 1965, Eguchi et al. 1966), mysids (Hallberg 1977), stomatopods (Schönenberger 1977), euphausiids

(Meyer-Rochow et al. 1978) and also in some insects (Home 1976, Meyer-Rochow 1971, 1972). Noteworthy is that several of these animals live in quite different environments, e.g. some mysids are deeper water forms and the insects are terrestrial.

Another case of convergent evolution is the eccentric cell of the terrestrial isopod Porcellio (Nemanic 1975, Nilsson unpublished). This cell also appears in the marine horseshoe crab, Limulus (Subphylum: Chelicerata) (Lasansky 1967, Miller 1957).

If, on the other hand, divergent evolution is considered, few examples exist. (Divergence is defined here as structural details of the eye from closely related taxa that become dissimilar in time.) One example is the fused continuous rhabdom (star-shaped in a cross section) of Idothea neglecta and Mesidothea and the fused layered rhabdom of the other investigated valviferan species. Also the rhabdoms of Porcellio and Oniscus differ in appearance, although both species live in the same terrestrial environment (Dahl et al. 1966). However, the suborder Oniscoidea is regarded as a heterogeneous taxon (Strömberg 1971) and evolutionary trends must be carefully evaluated here.

The different rhabdom types present in the various suborders of the Isopoda are summarized in Fig. 44.

In conclusion, owing to the varied appearance of the fine structure of the isopod eye, care must be taken when the eye is used in phylogenetic speculations. However, this difficulty can be circumvented if a large number of species are investigated and thorough functional analyses of the eyes are conducted.

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Figure legends

Fig. 1. The compound eyes of Cyathura carinata (light microscopy). Note the small eyes. Scale: 0.2 mm. X56

Fig. 2a. Frontal view of the head of Sphaeroma hookeri. Note the lateral position of eyes (SEM preparation). Scale: 0.3 mm. X30

Fig. 2b. Microvilli (arrows) located between the faceted lenses of Sphaeroma hookeri (SEM preparation). Scale: 2.5 μ m. X400

Fig. 2c. The compound eye of Sphaeroma hookeri. Note debris between the round lenses (see also Fig. 2b) (SEM preparation). Scale: 70 μ m. X140

Fig. 3. Frontal view of the head of Astacilla longicornis. Note the large and protruding eyes (SEM preparation). Scale: 0.3 mm. X30

Fig. 4. The head of Idothea viridis. Note the laterally positioned compound eyes (SEM preparation). Scale: 0.2 mm. X56

Fig. 5. Dorsal view of the head of Mesidothea entomon. Note dorsal position of the compound eyes (light microscopy). Scale: 0.4 mm. X25
Inset: Round facets roughly orthogonally packed. Scale: 0.1 mm. X50

Fig. 6. Frontal view of the head of Ligia oceanica. Note large and protruding eyes (SEM preparation). Scale: 0.5 mm. X22

Fig. 7. Two basically different types of organization of the delimiting membranes in the isopod compound eye. (a) basement membrane (BM); (b) fenestrated membrane (FM) and eye-capsule membrane (EM). long arrows indicate type of membrane in the different isopod sub-orders (underlined); open arrows indicate the region occupied by the retina; thick filled arrows (Diop./D) indicate the region occupied by the dioptric elements; asterisk the nuclear region of the retinula cells.

Fig. 8. Cross section of distally located crystalline cones in the eye of Sphaeroma hookeri. C corneagenous cell envelope (small black arrows nuclei); CC crystalline cone (large black arrows nucleus); RP reflecting pigment cell extension; white arrows suture line between the two main cone halves; open arrows accessory cone cells; asterisks nuclei of distal pigment cell. Scale: 3 μ m. X3,055. Inset: The peripherally located granular zone of the crystalline cone core (C) in the eye of Idothea viridis. PG pigment granule of a distal pigment cell; asterisk cone cell cytoplasm. Scale: 0.2 μ m. X19,500

Fig. 9. The fenestrated membrane in the eye of Idothea viridis. DP distal pigment cell end-feet distal to the membrane; RC retinula cell dendrite; arrows basal lamina of the end-feet; asterisk extracellular electron-dense substance. Scale: 1 μ m. X7,755

Fig. 10. Accessory cone cell elongation (arrow) terminating on the fenestrated membrane in the eye of Sphaeroma hookeri. The extension is encased by extracellular material (asterisk). DP end-feet of the distal pigment cell. Scale: 0.5 μ m. X28,200

Fig. 11. Schematic drawing of the eye-cup of Cyathura carinata. The cup is located below an undifferentiated cuticle (C) formed by a hypodermal cell layer (H). BM basement membrane which delimits the eye-cup; CC crystalline cone cores; GN glial cell nucleus; RH₁ longitudinal section of a rhabdom; RH₂ transverse section of the distal part of the rhabdom; RH₃ transverse section of the main portion of the rhabdom; asterisks indicate two other ommatidia with a deeper location than RH₁-RH₃; stippled area retinula cells.

Fig. 12. Transverse section of the eye of Cyathura carinata showing the eye-capsule, which is formed by a glial cell sheet (open arrow) and a basal lamina (filled arrow). MV microvilli of the rhabdom; PG pigment granule of the retinula cell; RC retinula cell. Scale: 1 μ m. X15,150

Fig. 13. Section of the bipartite crystalline cone (with two cone cores asterisks) of the eye of Cyathura carinata. H hypodermal cell; N nucleus either of a hypodermal cell or an accessory cone cell; arrow suture line between the two cone cells. Scale: 1 μ m. X7,510

Fig. 14. Section of the crystalline cone in the eye of Cyathura carinata. Note: two cone cores (asterisks) within the same cell. N nucleus of the crystalline cone cell. Scale: 0.5 μ m. X9,460

Fig. 15. Transverse section of the distally situated eighth rhabdomere (R8) of the eye of Cyathura carinata. MV microvilli of R8; arrows cell membranes between three other retinula cells. Scale: 1 μ m. X7,400

Fig. 16. Transverse section of the star-shaped rhabdom (RH) of Cyathura carinata. 1-7 retinula cells. Scale: 2 μ m. X2,990

Fig. 17. Part of a rhabdomere in the eye of Cyathura carinata. Opposing microvilli are separated by a thin retinular cell sheet (arrow). RC retinula cell. Scale: 0.5 μ m. X23,450

Fig. 18. Transverse section of the distal rhabdom in a light-adapted eye of Sphaeroma hookeri. DP distal pigment cell; RP reflecting pigment cell; 1-7 retinula cell; R8 retinula cell eight which has a distally situated rhabdomere (asterisk). Scale: 2 μ m. X2,925

Fig. 19. Schematic drawings of longitudinal sections of ommatidia in different isopod compound eyes. A Sphaeroma hookeri; B the eighth retinula cell of Sphaeroma, which bears a rhabdomere where opposing microvilli meet end-to-end (arrow), C Astacilla longicornis; D Idothea baltica; E Mesidothea entomon; F Ligia oceanica; G transverse section of the distal rhabdom of Ligia which shows the position of retinula cell eight (R8) between cells no. 1 and 7. A axon; AE accessory cone cell extension; C corneagenous cell; CC crystalline cone; CL cuticular lens; DP distal pigment cell; E eye-capsule membrane; G glial cell; MV microvilli; RC retinula cell; RH rhabdom; RP reflecting pigment cell; R8 retinula cell eight; arrows (in A, C, D, E, F) accessory cone cell; arrow (in B) opposing microvilli meet end-to-end; asterisk extracellular space.

Fig. 20. Transverse section of a rhabdom in a light-adapted eye of Sphaeroma hookeri. RH rhabdom; RPN nucleus of a reflecting pigment cell; 1-7 retinula cells; 8 axon of retinula cell eight; open arrows accessory cone cell extensions. Scale: 2 μm . X3,890
Inset: Opposing microvilli meet end-to-end (arrow). Scale: 0.5 μm . X7,755

Fig. 21. Oblique section of a rhabdom in a dark-adapted eye of Sphaeroma hookeri. RH rhabdom; RP reflecting pigment cell; 1-7 retinula cells; arrows accessory cone cell extensions. Scale: 2 μm . X4,550

Fig. 22. End-feet of a distal pigment cell in the dark-adapted eye of Sphaeroma hookeri. Arrow basal lamina of the fenestrated membrane; asterisk electron-lucent vesicle; star vesicle containing pigment-like granules. Scale: 1 μm . X12,900

Fig. 23. Bundle of retinula cell dendrites from an ommatidium in Sphaeroma hookeri. GN nucleus of a glial cell; RP extension of a reflecting pigment cell; stars the four large retinula cells; asterisks the three small retinula cells; 8 the eighth retinula cell. Scale: 2 μm . X2,440

Fig. 24. Axon bundle from one ommatidium in Astacilla longicornis. A axon; asterisk glial cell; arrow basal lamina. Scale: 1 μm . X7,470

Fig. 25. Light microscopical longitudinal section of the crystalline cones in the compound eye of Astacilla longicornis. CC crystalline cone; DP distal pigment cell layer; heavily pigmented, filled arrows nuclei of the distal pigment cells; open arrows retinula cell nuclei; large white arrow nucleus of a crystalline cone cell; small white arrow nucleus of an accessory cone cell; asterisk rhabdom. Scale: 20 μm . X395

Fig. 26. Longitudinal section of the region of the retinula cell nuclei in the eye of Astacilla longicornis. DP heavily pigmented layer of distal pigment cells; N nuclei of the retinula cells; RP reflecting pigment cell; RC retinula cell; arrows unpigmented processes emerging from the soma of the retinula cells. Scale: 0.5 μm . X1,365

Fig. 27. Transverse (slightly oblique) section of the rhabdom in the compound eye of Astacilla longicornis. Note the orthogonally oriented microvilli (arrows) and the pronounced extracellular palisade (asterisk). RH rhabdom; 1-6 retinula cells; open arrows accessory cone cell extensions. Scale: 1 μm . X5,750

Fig. 28. The basement membrane in the eye of Astacilla longicornis. The end-feet of the distal pigment cell (DP) contain intracellular densities (asterisk). RC retinular cell; black arrows basal lamina; open arrows glial cell sheet. Scale 2 μm . X4,940

Fig. 29. Transverse section of the distally located rhabdomere of retinula cell seven (R7) in the eye of Idothea baltica (light-adapted) MV microvilli of the seventh rhabdomere; RP reflecting pigment cell; open arrows microvilli of the main rhabdom. Scale: 2 μ m. X5,610

Fig. 30. Transverse section of the rhabdom (RH) of the light-adapted eye of Idothea baltica. RP reflecting pigment cell; 1-6 retinula cells arrows accessory cone cell extension. Scale: 2 μ m. X3,500

Fig. 31. Transverse section of a rhabdom in a dark-adapted compound eye of Idothea baltica. Note thin retinula cells and expanded reflecting pigment cells. RH rhabdom; RP reflecting pigment cell (see also Fig. 30). Scale: 2 μ m. X3,625

Fig. 32. Longitudinal section of the fused layered rhabdom in the compound eye of Idothea baltica. MV microvilli. Scale: 1 μ m. X8,875

Fig. 33. Longitudinal, light microscopical section, of the rhabdom in the eye of Idothea emarginata. Note the wavy appearance at the rhabdom (RH) periphery. RC retinula cell. Scale: 10 μ m. X1,050

Fig. 34. Transverse section (slightly oblique) of the rhabdom (RH) in the eye of Idothea neglecta (light-adapted). RP reflecting pigment cell; 1-6 retinula cells; arrows accessory cone cell extensions. Scale: 2 μ m. X4,400

Fig. 35. Large granules in the retinula cell cytoplasm in the eye of Idothea neglecta. GR granule; open arrow typical electron-dense granules of the retinula cells. Scale: 1 μ m. X5,800

Fig. 36. The eye-capsule membrane in Idothea neglecta. RC soma of the retinula cell; arrow glial cell; asterisk extracellular space. Scale: 5 μm . X1,880

Fig. 37. Transverse section (medially) of the star-shaped rhabdom in the compound eye of Mesidothea entomon. RH rhabdom; 1-7 retinula cells. Scale: 4 μm . X2,400

Fig. 38. Transverse section (medially) of the small seventh rhabdomere (R7) in the compound eye of Mesidothea entomon. Arrow glial sheet between retinula cells. Scale: 2 μm . X4,760

Fig. 39. An aberrant cell in the retinula cell nuclei region in the compound eye of Mesidothea entomon. Note: large amount of intracellular membranes (asterisk). N nucleus of aberrant cell; RCN retinula cell nucleus; filled arrows glial cell; open arrows pigment granules. Scale: 3 μm . X2,875

Fig. 40. The cytoplasmic portion of the proximal cone in the eye of Ligia oceanica. Arrow suture line between the two cone cells. Scale: 1 μm . X7,755

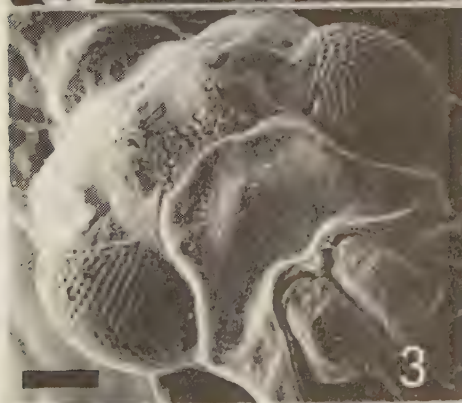
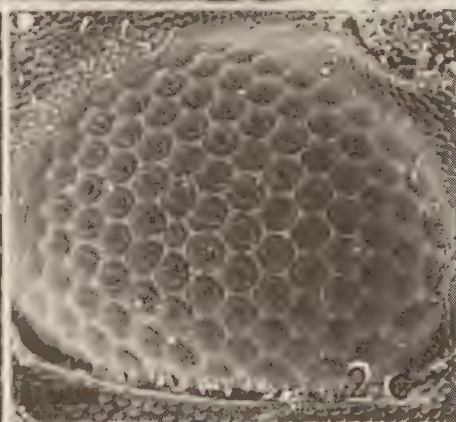
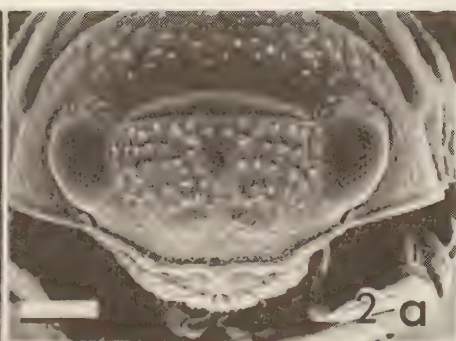
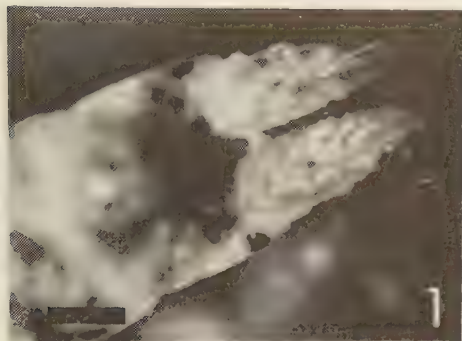
Fig. 41. Longitudinal light microscopical section of the crystalline cone in the eye of Ligia oceanica. CC dense core of the distal portion of the cone; DP distal pigment cell; asterisk cytoplasmic portion of the proximal cone; open arrow rhabdomere of retinula cell eight; white arrow corneagenous cell. Scale: 1 μm . X900

Fig. 42. Cross section of the distally situated eighth retinula cell in the eye of Ligia oceanica. MV microvilli; RC retinula cell; asterisks crystalline cone cells; 8 retinula cell no. 8; arrow corneagenous cell. Scale: 2 μ m. X3,960

Fig. 43. Longitudinal section of the proximal rhabdom in Ligia oceanica. Between the separated rhabdomeres a reflecting pigment cell (RP) is present. MV microvilli of a rhabdomere; RC retinula cell; RP reflecting pigment cell; open arrow a cluster of granules of the reflecting pigment cell. Scale: 2 μ m. X4,210

Inset: Reflecting pigment cell granules. Scale: 0.5 μ m. X7,755

Fig. 44. Schematic drawings of the varied morphological appearance of the crystalline cones and rhabdoms in the compound eyes of different isopod suborders (underlined). Arrows indicate morphological types present in the different suborders. a vestigial cones (see also 2); b pyriform or spherical cones; c elongated cones; d bipartite cone which comprises a distal electron-dense core and a proximal cytoplasmic part. A fused continuous rhabdom (star-shaped in a cross section); B semi-fused rhabdom (longitudinal section); C fused layered rhabdom (longitudinal section); D fused continuous rhabdom (round, oval or rectangular in a cross section); E fused continuous rhabdom which possesses an eccentric cell in the centre of the rhabdom (non-stippled area). 1, the cross-sectional shape of the same rhabdom varies in the distal and proximal part. 2. According to Martin (1976), the epicarid Athelges possesses a rhabdom with orthogonally oriented microvilli. Generally the lateral eyes of epicarids are vestigial. ? No reports on eye structure are known.



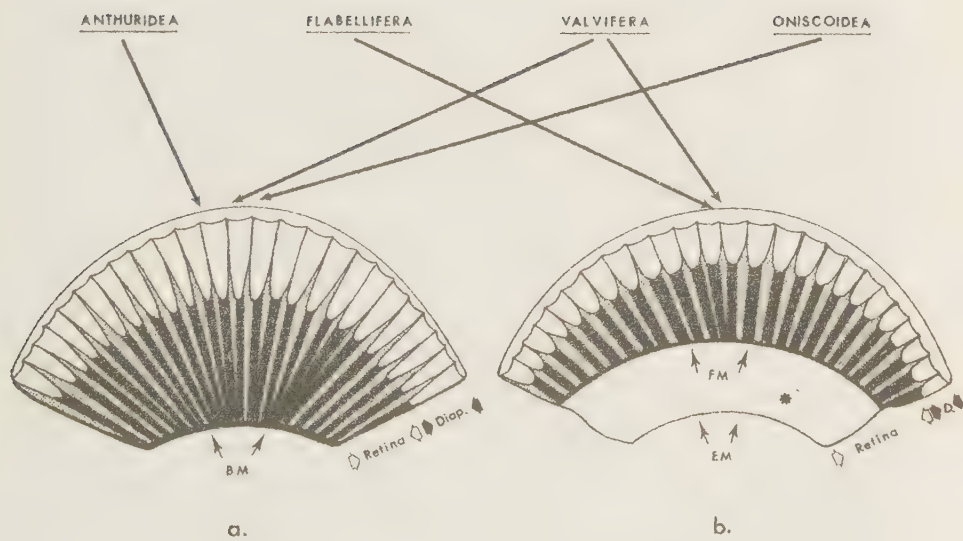
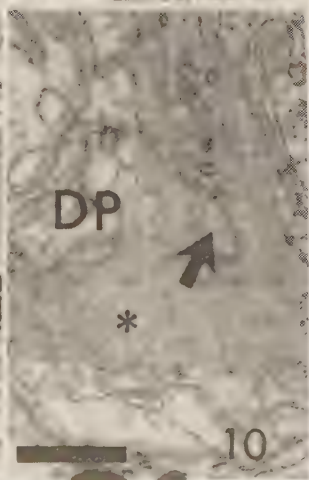
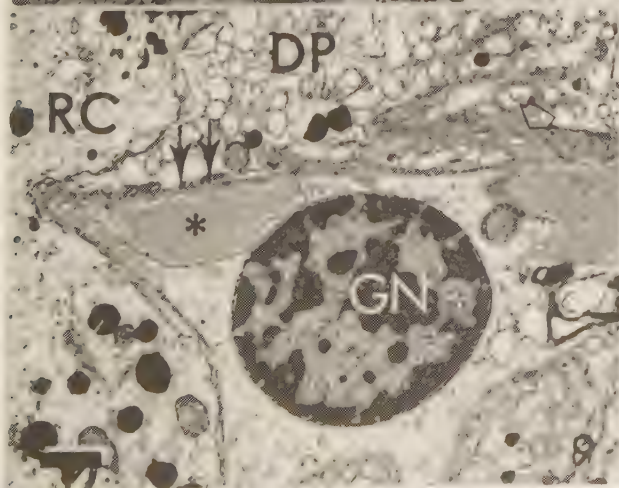
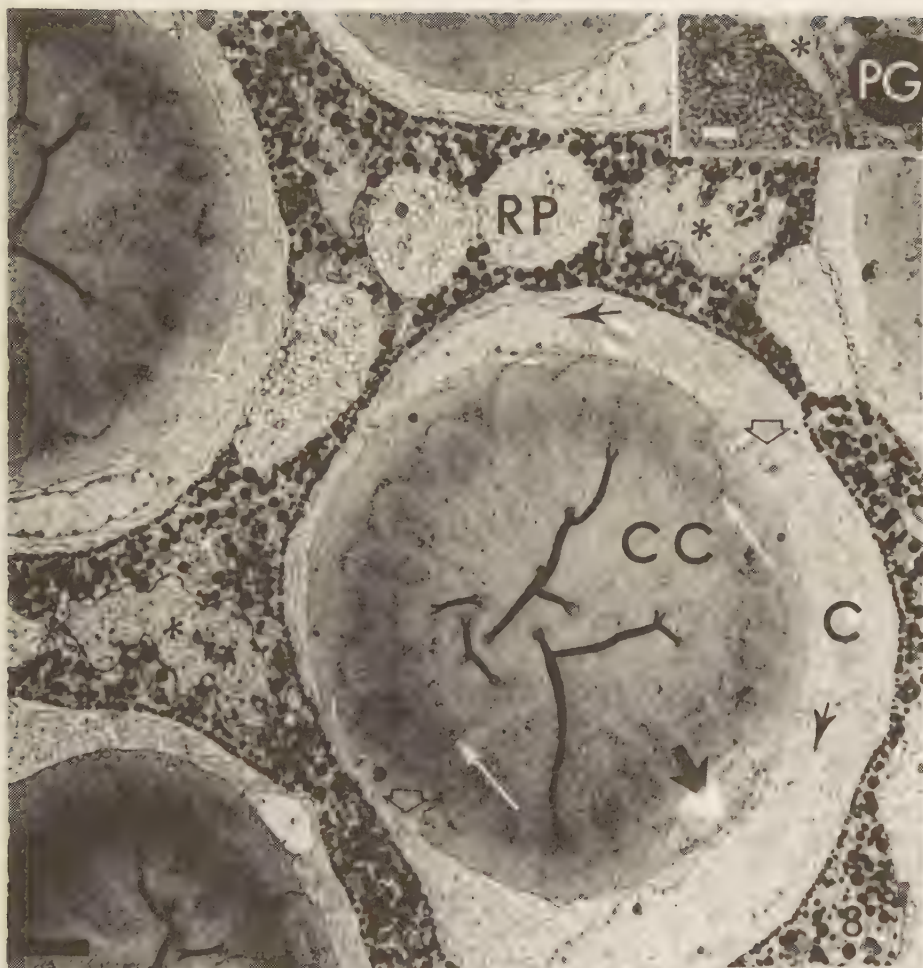


Fig. 7.



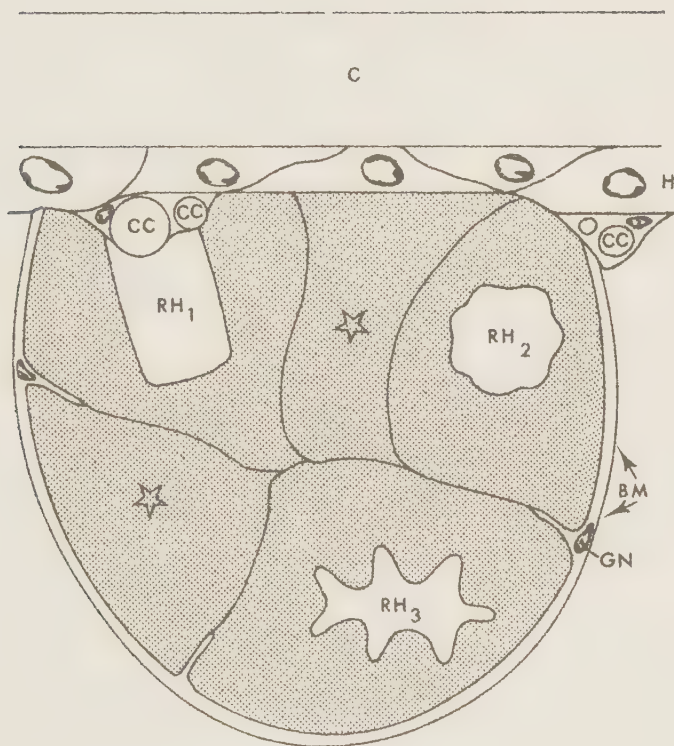
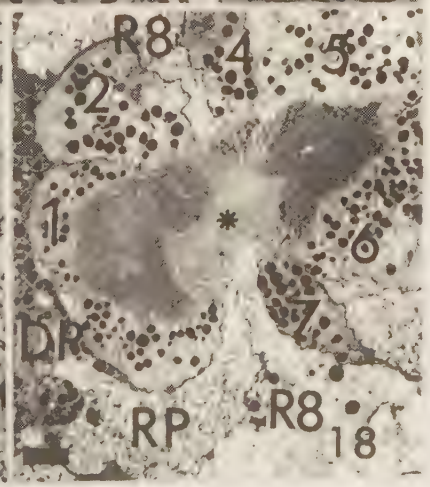
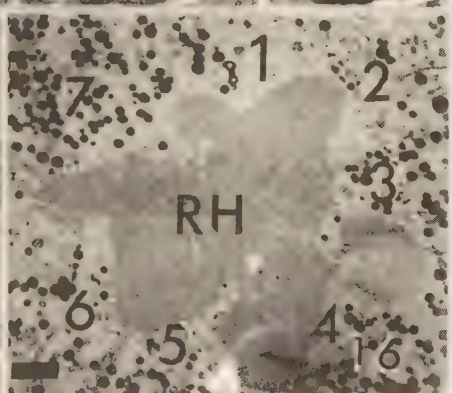
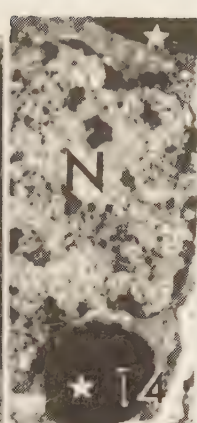
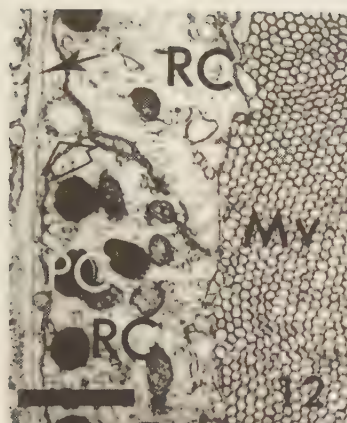


Fig. 11.



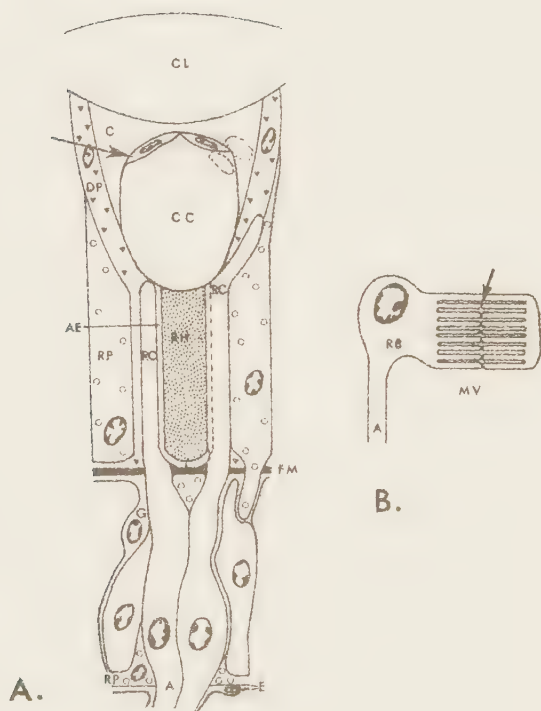


Fig.19.

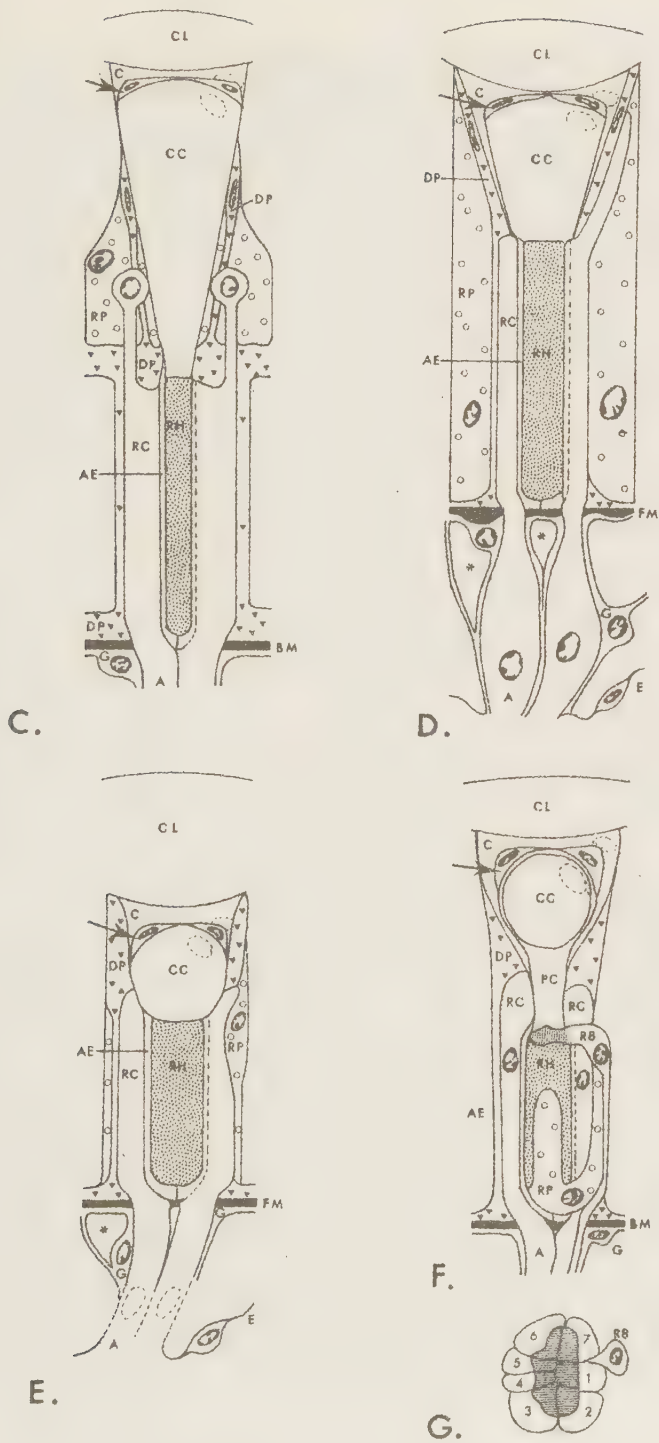
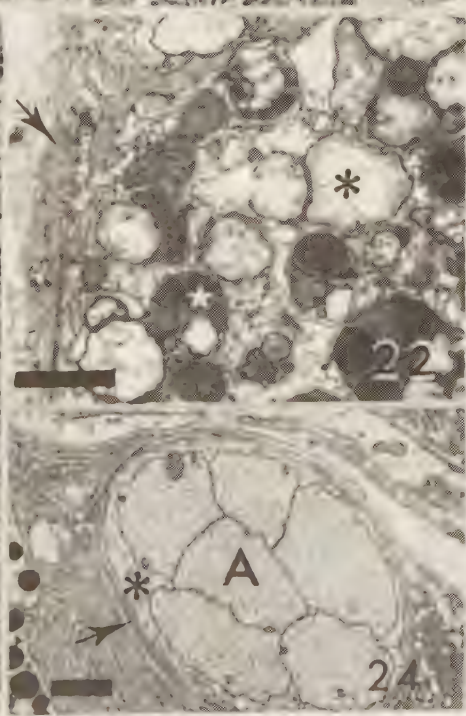
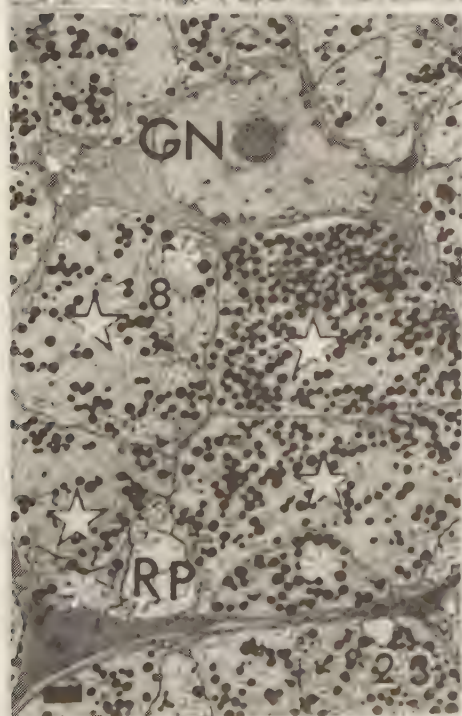
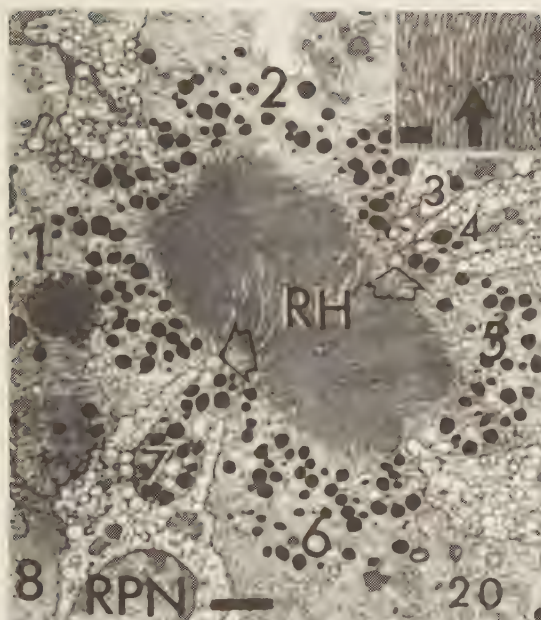
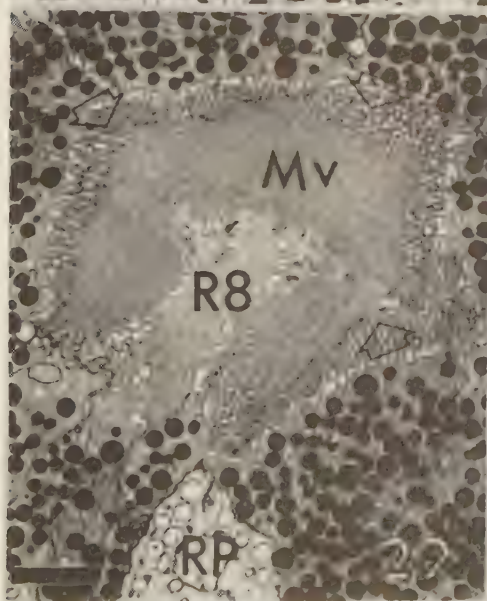
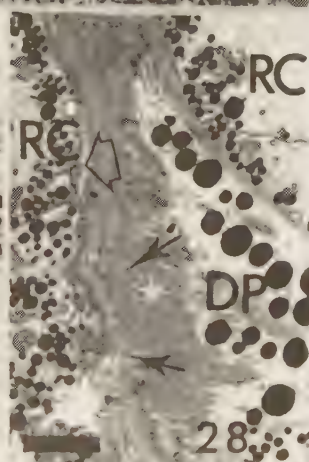
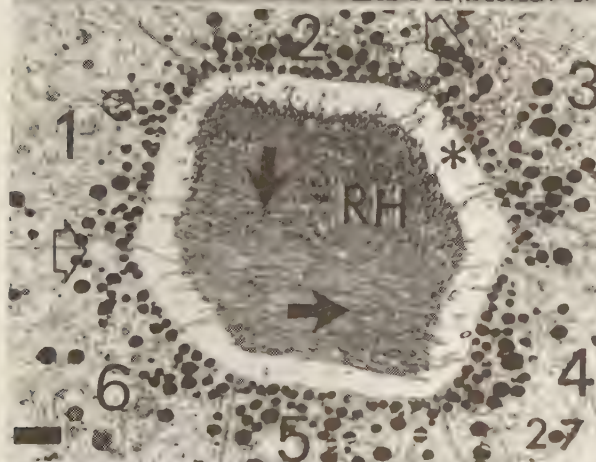
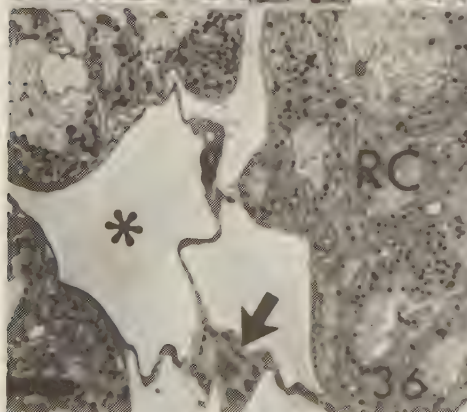
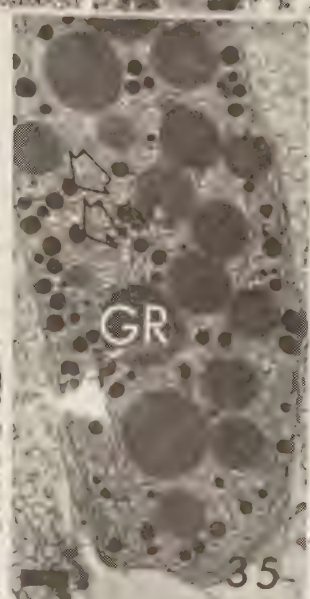
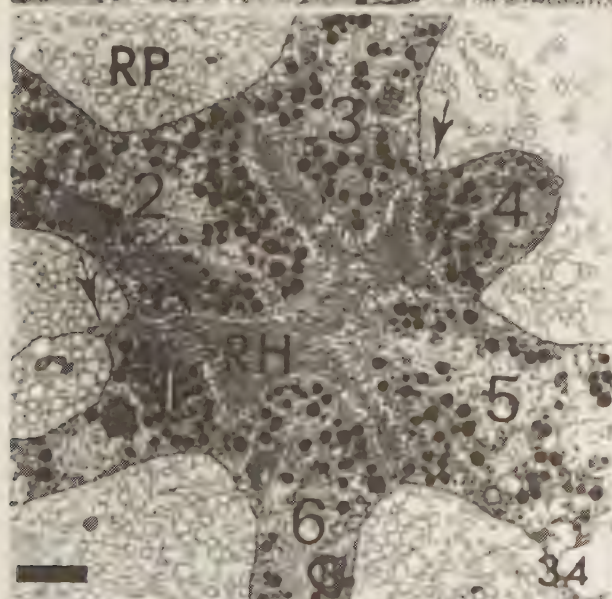
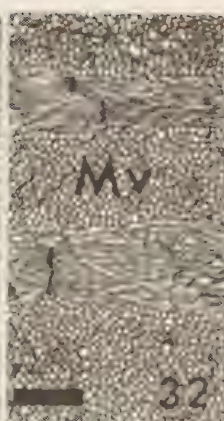
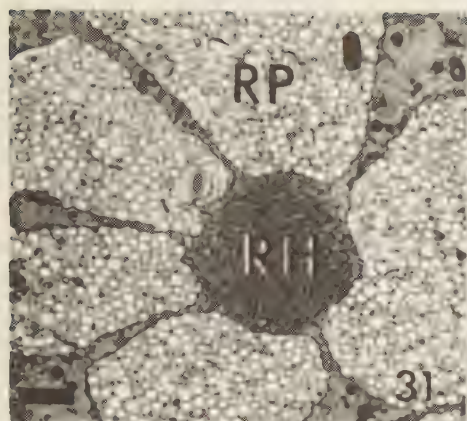
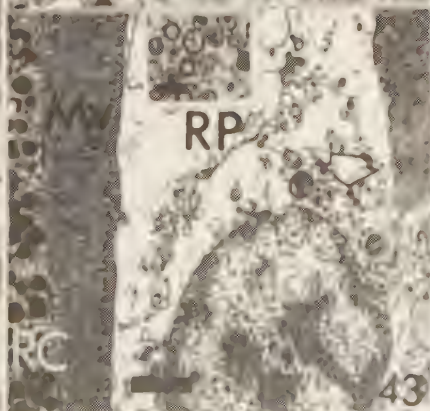
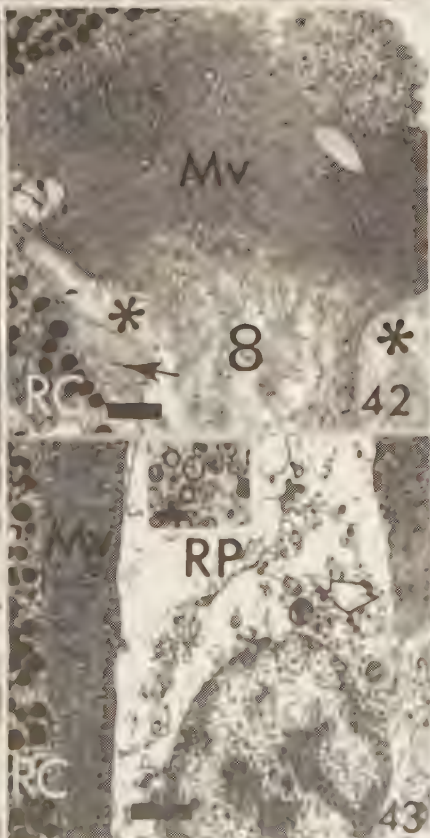
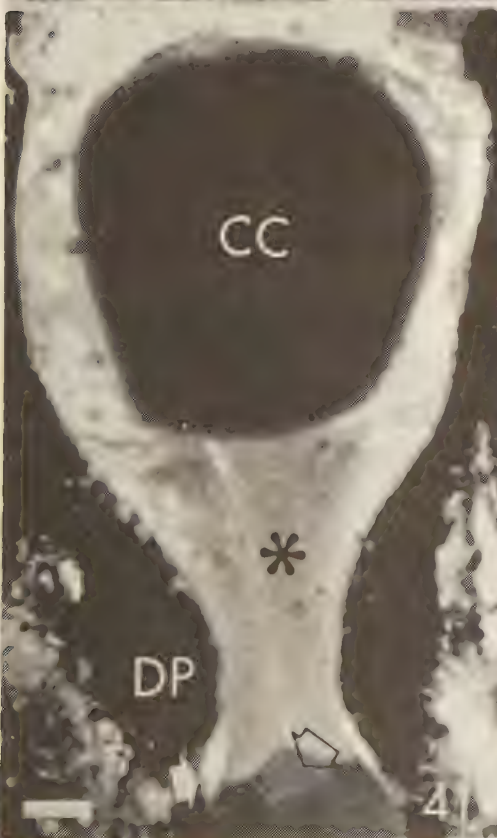
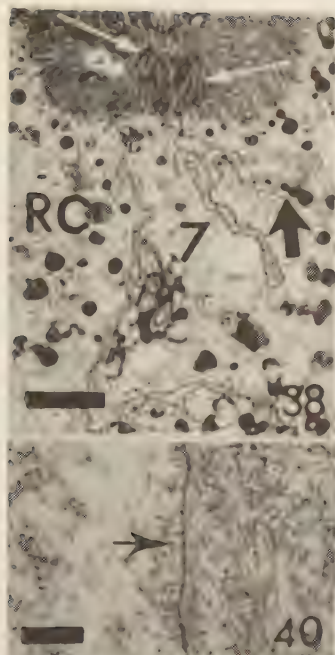


Fig. 19.









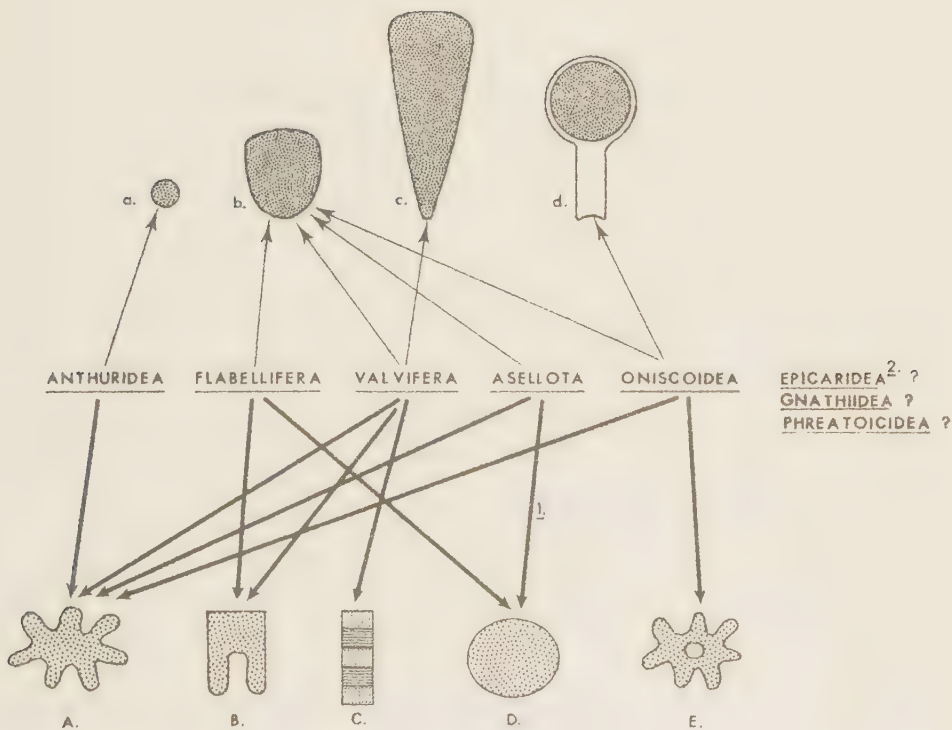


Fig. 44.

Fine Structural Changes Caused by Daylight Exposure of
the Compound Eye of Cirolana borealis (Crustacea)

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Running title: Fine Structure and Daylight Exposure of
the Cirolana Compound Eye

Key words: Crustacea — Anatomy — Compound eye — Retinal damage

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Summary: The effect of light on the compound eyes of the deep-water-inhabiting crustacean isopod Cirolana borealis Lilljeborg was investigated. Animals were captured and fixed at night (DA = dark-adapted) and day (LE = light-exposed), respectively. Each eye comprises about 60 ommatidia with large lenses (diam. c. 150 μm), spherical crystalline cones, and hypertrophied rhabdoms. The cones are formed by two main cone cells, and in addition two accessory ones are present. Seven retinula cells contribute to the rhabdom, which is fused distally and open proximally. Reflecting pigment cells form a tapetum and distal pigment cells screen neighbouring ommatidia. The anatomy of the light-exposed eyes differs from the dark-adapted eyes as follows: the microvilli are disrupted; retinula cell pigment occurs in the rhabdom; the retinula cell cytoplasm is vesiculated. The results have been interpreted as retinal damage caused by excessive light.

Introduction

Excessive light is known to negatively effect photoreceptor membranes, causing disrupted microvilli in some arthropods (Behrens and Krebs 1976; Tuurala and Lehtinen 1971) and in slugs (Eakin and Brandenburger 1974). This might be true of particularly deep-sea-inhabiting species where normal daylight has been found to be harmful to the eyes (Loew 1976; Meyer-Rochow 1981).

The marine crustacean isopod Cirolana borealis, living in rather deep marine waters, has compound eyes with anatomical specializations, as hypertrophied rhabdoms and large lenses (Beddard 1888; Hesse 1901; Parker 1891), that suggest an adaptation to dim-light environments (Nilsson and Nilsson 1981). Thus, these eyes seem prone to react to excessive light by a destruction of especially the microvilli of the rhabdom. This light-induced destruction must, however, be separated from fixation artifacts, and hence these two parameters have been investigated.

In addition, the anatomy of the compound eye of Cirolana, known from earlier light microscopical investigations (Beddard 1888; Hesse 1901; Parker 1891) differs from other isopod eyes analysed in more recent years with the electron microscope (Edwards 1969; Nemanic 1975; Nilsson 1978; Nilsson and Elofsson 1977) in several respects, e.g., as regards the structure of the crystalline cone, the number of receptor cells and the presence of "hyaline cells". A re-investigation of the eye, especially the fine structure, was deemed necessary.

Materials and Methods

The crustacean isopod Cirolana borealis Lilljeborg was trapped in cages baited with fresh fish, at a depth of 100 metres, in Raunefjord off Bergen, Norway.

Animals were obtained during the daytime and at night. Daylight animals were kept in daylight for at least 1 h, or for several days in room light in an aquaria room, and fixed in daylight. Night-captured animals were kept in darkness and fixed in darkness, either at night, at dusk, or in daytime. Dissection was performed in red-safe light (Philips E27 PF 712 E) and the procedure did not exceed 5 min. Three fixatives were used for fixation of half heads: 2.5% glutaraldehyde, 1% OsO_4 , and paraformaldehyde/glutaraldehyde according to Karnovsky (1965, slightly modified; Ca^{2+} omitted) at 4°C , pH 7.2-7.4, for 3-4 h (1% OsO_4 for 2 h). Sodium cacodylate buffer (0.2 M, 400 m Osmol) was used throughout.

Postfixation was carried out for 2 h in 1% OsO_4 (except the osmium-fixed eyes). Dehydration was performed in an alcohol series, blockstaining in 1% PTA and 0.5% UA, embedding in Vestopal W. Ultrathin sections were placed on formvar-coated grids and examined in Zeiss EM 10 A and Jeol 100 CX electron microscopes. Sections, 2 μm thick, for light microscopy were mounted on glass and stained with azure-methyl (Richardson et al. 1960).

Results

General Morphology

The location of the eyes on the head segment is illustrated in Fig. 3. About 60 ommatidia comprise the compound eye of an adult animal. The ommatidium has a well-developed dioptric apparatus, a hypertrophied and compact rhabdom, which is fused distally and open proximally. The rhabdom is formed by seven retinula cells. In addition, reflecting and distal pigment cells are present. The eye rests on a double basement membrane.

Dioptric Elements

The biconvex corneal lenses are strongly curved internally, whereas externally the curvature is less prominent and the hexagonal facet pattern is not observable on the eye surface. The central areas of the eye have larger facets, and the facet diameter decreases successively towards the periphery. The central and peripheral thicknesses of the central lenses are 55 and 20 μm , respectively. The cornea has prominent endocuticular lamellae that confer in a distinct layered appearance when observed in sections. The lenses are formed by two corneagenous cells, which have large nuclei each with a prominent nucleolus. The cytoplasm contains what is here considered to be large artifactual vacuoles (Fig. 2). The corneagenous cells extend as an envelope around the crystalline cone. Distally, the envelope is 15 μm thick and it tapers to a proximal diameter of 2 μm .

In other investigated isopods, this envelope is considerably thinner than in Cirolana (Edwards 1969; Nemanic 1975; Nilsson 1978). The round crystalline cones are flattened distally, possibly ensuring better contact with the inner curvature of the lens. The ultrastructure of the cone is similar to other isopods investigated (Edwards 1969; Nemanic 1975; Nilsson 1978): there is an inner amorphous core (90-100 μm in diam.), a thin granular zone surrounding the core (2 μm thick), and peripherally a thin envelope (1 μm thick) of cone cell cytoplasm. The cone is formed by two main cone cells and in addition two accessory ones are present distally, located at the end points of the suture-line dividing the two cone halves (Figs. 1, 4).

The Retina Dark-adapted Eyes (DA)

The seven retinula cells in each ommatidium have large and elongated nuclei that contain small amounts of heterochromatin. These cells are situated at the level of the distal part of the rhabdom. Electron-opaque pigment granules (diam. about 0.3 μm) are present in the retinula cell cytoplasm and resemble ommochromes (Elofsson and Hallberg 1973) (Fig. 9). A thin layer (less than 1-2 μm thick) of retinula cell cytoplasm, with few organelles, surrounds the rhabdom and somewhat thicker sheets (folds) project into the rhabdom area (Fig. 9, see below).

Proximal to the rhabdom, the retinula cells expand and are characterized by a large number of mitochondria (Fig. 13). The retinula cells penetrate a basement membrane and proximal to this

the axons penetrate a second delimiting membrane of the eye (see below).

The rhabdom is formed by seven identical rhabdomeres (Figs. 1, 6). The rhabdom is an oval, compact cylindric-shaped structure, about 100 μm in diameter and 85 μm in length, that is fused distally (Fig. 6). The proximal portion of the rhabdom is split, with the rhabdomeres (diam. 35 μm , length 35 μm) separated by reflecting pigment cells (Figs. 1, 9). This rhabdom type is designated as semifused (Nilsson and Nilsson 1981). Each rhabdomere is formed by four to six retinular cell folds protruding from the cell wall. Microvilli originate, at right angles, from two opposite folds and meet ends on forming microvillar "lamellae" (Figs. 1, 6, 9). In transverse sections the lamellae resemble the spokes of a wheel. Frequently the lamellae are dichotomously split (Figs. 1, 6, 9).

The microvilli are 60 nm thick except for the most peripherally located ones, which are about 100 nm thick (Fig. 8). The contour of the microvilli is smooth, and no indications of dilatations and disruptions are present as in light-exposed eyes (see below). The microvilli are tightly stacked and the extracellular space is small (Fig. 8 inset). Each microvillus possesses a central filament, c. 10 nm thick (Fig. 8 inset), that is confined to the microvillus in contrast to the isopod Astacilla (Nilsson and Elofsson 1977) and the amphipod Phronima (Ball 1977), where the filament continues into the retinula cell cytoplasm.

Screening Pigment Cells

Three types of accessory pigment cells are present: distal pigment cells, proximal reflecting pigment cells, and a new third type. The distal pigment cells have their lobed nuclei immediately below the cornea and the cells continue as a heavily pigmented envelope around the crystalline cones and the rhabdoms. Proximal to the rhabdom, the cells divide into a number of processes and distal to the basement membrane they expand and unite into a heavily pigmented layer, 10 μm thick. Each ommatidium is surrounded by six cells. The cytoplasm of the distal pigment cell is densely stacked with electron-opaque pigment granules representing two size classes, 0.2 μm and 0.4 μm , respectively. These granules have the ultrastructural characteristics of ommochromes (Elofsson and Hallberg 1973). In addition, granules have a different regional distribution: The smaller ones are present distally, are arranged in two to five layers (appear in sections like pearls on a string), and are always close to the crystalline cone (Fig. 5). The larger granules are located in the central portion of the cytoplasm and proximally are the predominating granule type (Fig. 15). A 4 μm -thick reflecting pigment layer (in reflecting light, golden yellow) is arranged like a bowl around the thin sheet of retinula cell cytoplasm of the rhabdom. Extensions isolate the separated proximal rhabdomeres. Proximal to the rhabdom, the reflecting cell layer forms a 60 μm -thick layer reaching down to the extended part of the distal pigment cell layer above the basal lamina (Figs. 1, 15). The membrane-bound granules have a diameter of 0.4 μm and appear "empty" (Fig. 12). Elongated mitochondria

(4-5 μm long), smooth endoplasmic reticulum, vacuoles, and conspicuous Golgi apparatuses are frequently found (Fig. 12). The third new type of pigment cell has no definite position and can be situated distally or proximally. Usually, it is found near the crystalline cone. The cell is characterized by electron-translucent granules (diam. c. 0.5-0.6 μm) resembling carotenoid pigment granules (Fig. 14, Elofsson and Hallberg 1973).

The Basement Membrane

Proximally, the eye is delimited by two well-developed membranes: a basement membrane and proximal to this a membrane of similar structure. The basement membrane is composed of an extracellular fibrillar basal lamina and a cellular component originating from glial cells, located proximally (Figs. 13, 15). Distal pigment cells rest on the membrane and possibly contribute to it. The second membrane is formed by the same glial cells and is similar in appearance (Fig. 15). The two membranes separate a 10-20 μm wide space within which the retinula cell axons run after penetrating the first membrane. They penetrate the second membrane at regular intervals and enter the first optic ganglion which is located immediately below. The axons are encapsulated by the glial cells in a mesaxonal arrangement and are contained in bundles of seven.

Thin processes from the glial cells extend distally through the basement membrane and envelop the axons when passing through

the basal pigment screen (Figs. 1, 13). Some processes extend to the proximal region of the rhabdom where they ramify and "bind" together the proximal tips of the open rhabdomeres (Fig. 1).

Fixation Experiments

The results using three different fixatives (2.5% glutaraldehyde, 1% OsO_4 , and paraformaldehyde/glutaraldehyde according to Karnovsky (1965)) on night eyes were that the two first fixatives yielded swollen microvilli, distended ER, and retinula cell pigment granules migrating into the rhabdom, whereas none of these were found in the preparations fixed according to Karnovsky (1965).

Light-exposed Eyes (LE)

Upon exposure to daylight or aquaria room light morphological changes appear in the compound eye that involve the retinula cells. These changes are compared here with night eyes fixed according to Karnovsky (1965).

The microvillar lamellae of the rhabdom lose their orderly arrangement and no specific microvillar orientations can be found for the rhabdomeres (Figs. 7, 11). The microvilli are seldom well preserved; instead, they are either dilatated, giving them a swollen and bulbous appearance, or shrunken and then tightly packed (Fig. 11). Frequently, they collapse and form aggregates of fused membranes, phagosomes (Fig. 11 inset).

At the microvillar bases a large number of double-membraned vesicles are present (putative microvillar membranes). Larger single-membraned vesicles, extended ER, are found in the retinula cell cytoplasm (Fig. 10). Multivesicular bodies are occasionally found, although they are not abundant. The general preservation of the cytoplasm and its organelles is poor.

A characteristic feature of the light-exposed eyes is the presence of pigment granules in the cytoplasmic folds within the rhabdom area. The granules are irregularly distributed and are located from the periphery to the centre of the rhabdom (Fig. 7). Eyes obtained from animals kept in the darkness from one to several days were fixed at dusk, at night and at midday. No endogenous rhythm in the turnover of membranes was found.

Discussion

The results are discussed in two subsections; the first deals with the anatomy compared with other isopods and the second with structural changes caused by light exposure.

Anatomy

The gross structure of the compound eye of Cirolana borealis differs in the following details from older light microscopical investigations (Beddard 1888; Hesse 1901; Parker 1891): in the

structure of the crystalline cone, in the presence of distal pigment cells, in the interpretation of the so-called hyaline cells, and in the bounding membrane delimiting the eye. The crystalline cones are formed by two main cone cells and in addition two accessory cone cells are present. The occurrence of the latter in Cirolana, as well as in other investigated isopods (Nilsson 1978) and mysids (Hallberg 1978), adds to the evidence of a common building plan of the dioptric apparatus.

The heavily pigmented envelope around the crystalline cones, and also between neighbouring ommatidia, originates from distal pigment cells and not from retinula cells, as reported previously (Beddard 1888). Distal pigment cells occur frequently within the isopoda (Debaisieux 1944).

Extensions from the distal pigment cells extend to the basement membrane, and this is similar to the isopod Jaera albifrons (Nilsson 1978). However, the extensions of the latter are sparsely pigmented in contrast to those of Cirolana. The significance of the extensions in Cirolana is twofold. First, they may participate in the formation of the basement membrane (see below); and secondly they make possible the formation of a heavy screening pigment layer proximal to the rhabdoms (or actually proximal to the tapetal layer) replacing heavily pigmented retinula cells present in other isopods (Edwards 1969; Nemanic 1975; Nilsson 1978). Another example of accomplishing a proximal pigment screen is exemplified by the basal pigment cells found in other crustacean groups (Hallberg 1978).

The reflecting pigment cells of Cirolana were previously thought to be a special cell type, so-called hyaline cells,

exclusively found in the flabelliferan isopods (Beddard 1888). Two cells were found in each ommatidium, but this number disagrees with the present investigation, where only one is present. The transparent appearance of these cells referred to by light microscopist must be attributed to leakage or elution of the pigment granules during the histological procedure.

The two membranes delimiting the eye in Cirolana are strikingly similar to the fenestrated membrane and the eye-capsule membrane of other isopods investigated (Nilsson 1978). These membranes are formed by an acellular basal lamina and a cellular component, glial cells (homologues to the basal cells of Jaera albifrons and Asellus aquaticus (Nilsson 1978)).

In Jaera the distal pigment cells terminate on the membrane and contribute to its formation; and in Cirolana a similar arrangement is present, although the contribution of the distal pigment cell is less conspicuous. The main difference in Cirolana compared with other investigated isopods is the position of the membranes in relation to the retinula cell nuclei. The so-called fenestrated membrane is distal to the nuclei (definition according to Peabody 1939) and the eye-capsule membrane delimits the eye peripherally, whereas in Cirolana both membranes are proximal to the retinula cell nuclei. However, the fine structure of the membranes and the way they are formed and also their number make it probable that these membranes are homologues to the fenestrated membrane and the eye-capsule membrane. Not only the isopods, but also the amphipods (Hallberg et al. 1980) have the same organization of the delimiting membranes of the eye. This can also be extended to include all peracarid crustaceans apart from mysids, as has been shown by Odselius and Elofsson (1981).

The division of large rhabdoms of Cirolana into a distally fused portion and a proximally open portion is found in the isopod suborder Flabellifera, and in addition to Cirolana it has been described in Aega (Beddard 1888). One case has also been reported in the valviferan isopod Ligia oceanica (Edwards 1969). Other isopods investigated have fused continuous rhabdoms (for review, see Nilsson 1978). The functional implications of this rhabdom type in Cirolana have been suggested by Nilsson and Nilsson (1981).

The presence of seven retinula cells in Cirolana is in contrast to other isopods investigated. A distally positioned aberrant eighth receptor cell is present in the isopod Porcellio (Nemanic 1975), in Jaera and Asellus (Nilsson 1978), and a basal one is found in Ligia (Edwards 1965). The latter cell has no rhabdomere. The number of receptor cells, the hypertrophied rhabdom, and the tapetum indicate that Cirolana has a compound eye structure that differs from that of other isopod groups. Cirolana has a pronounced enlargement of the photopigment-bearing membranes, and this is also the case in some deep-sea crustaceans, like mysids (Elofsson and Hallberg 1977) and amphipods belonging to the family Lysianassidae (Hallberg et al. 1980). The Cirolana eye is, however, less simplified (when considering structural elements) than those of the latter two groups of crustaceans. These, for instance, totally lack dioptric elements and have but one type of pigment cells — red or reflecting pigment cells.

Light Exposure

Structural changes, like disorganization and swelling of the rhabdomeric microvilli, result from regulated cyclic events (Bedini et al. 1977; Blest 1978; Stowe 1980) or fixational artifacts (Williams 1980) or by different agents, such as vitamin A deficiency (Carlson et al. 1969), prolonged exposure to total darkness (Eakin and Brandenburger 1974; Edwards 1969; Eguchi and Waterman 1966, 1979; Nemanic 1975; Roach and Wiersma 1974; White 1967), and exposure to bright light (Behrens and Krebs 1976; Loew 1976; Meyer-Rochow 1981; Tuurala and Lehtinen 1971).

To rule out the influence of a poor histological preservation, different fixatives were used. Excellent preservation was obtained by the combined glutaraldehyde/paraformaldehyde fixation of Karnovsky (1965). Using this, other parameters were investigated. No endogenous rhythm could be found in permanent darkness based on the appearance of the rhabdom of the retinular cells, nor were any screening pigment movements observed. Eyes exposed to daylight more than 1 h or room light for several days showed an altered morphology compared with night-adapted eyes. These changes were restricted to the retinula cells and their rhabdomeres and the changes are: disordered and swollen microvilli, a poor histology and vesiculation of the cytoplasm and pigment granules within the rhabdom. Similar light-induced changes, excluding the pigment migration, have been reported in the deep-sea crustacean Nephrops, where the change is irreversible (Loew 1976)

and in the amphipod Orchomene, living under the Ross Ice Shelf (Meyer-Rochow 1981). Changes of this kind are likely to render the eyes useless in bright light -- a memento to workers studying the behaviour of similar animals.

The eyes of Cirolana are adapted to low light intensities (Lindström and Nilsson 1982; Nilsson and Lindström 1982; Nilsson and Nilsson 1981) and it is shown here that the eyes, when exposed to bright light, lack clear mechanisms for protection of the eye as has been shown in, for example, other crustaceans, Grapsus (Nässel and Waterman 1979) and Leptograpsus (Stowe 1980). Whether the pigment migration into the rhabdom is a true adaptive mechanism at the ambient light intensities of the animal is unknown at present. On preservation of such eyes for histological purposes, care has to be taken to find a suitable fixative because apparently the retinula cells are more susceptible to fixation than eyes adapted to normal light.

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Figure Legends

Fig. 1. Schematical drawings of the ommatidium of Cirolana borealis.

a. Longitudinally sectioned ommatidium; b and c indicate illustrated levels seen in corresponding figures, b. cross section at the level of the dioptric apparatus, c. a partly three-dimensional presentation of the lamellar arrangement of the microvilli of a rhabdomere; upper arrow cross section of the distally fused rhabdom illustrated in d, lower arrow cross section of the proximally open rhabdom illustrated in e. A axon; ACC accessory cone cell; BL basal lamina; BM basement membrane; C corneagenous cell; CC crystalline cone cell; CO corneagenous lens; DP distal pigment cell; E eye capsule membrane; G glial cell; glial cell process; MV microvillar lamellae; R rhabdomere; RC retinula cell; RP reflecting pigment.

Fig. 2. Transverse section of the dioptric elements. C corneagenous cell; CC crystalline cone cell; M mitochondria; arrows indicating zone between cytoplasmic and granular portion of the crystalline cone. Scale 1 μm

Fig. 3. The dorsal side of the head in Cirolana borealis (SEM). Arrow the left compound eye (irregular shapes and depression of ommatidia are artifacts, here visualizing the facet pattern otherwise obscured by the smooth surface of the eye cuticle). Scale 500 μm . Inset: View from the ventral side of the head, showing laterally and ventrally situated ommatidia. Scale 500 μm

Fig. 4. Transverse section of dioptric elements showing the accessory cone cell. ACC accessory cone cell; CC crystalline cone cell; arrow suture-line between the two main cone cell halves. Scale 1.5 μm

Fig. 5. Transverse section of a distal pigment cell in the region of the crystalline cone. Note the layered arrangement of the small pigment granules close to the cone (up in picture) and the irregular arrangement of larger granules peripherally in the cell (down in picture). Scale 1 μm

Fig. 6. Light-microscopical transverse section showing fused rhabdomeres distally (dark-adapted eye). RH rhabdom; arrows retinula cell pigment granules outside the rhabdom; asterisks reflecting pigment cell layer around the rhabdom. Scale 35 μm

Fig. 7. Light-microscopical transverse section showing fused rhabdomeres distally (light-exposed eye). Arrow retinula cell pigment granules within the rhabdom; asterisks distal pigment cell layer around the rhabdom. Scale 35 μm

Fig. 8. Longitudinally sectioned microvilli meeting end to end (small arrows) microvillar lamellae. Thicker microvilli at the base of the lamellae (large arrow). Dark-adapted eye. Scale 0.5 μm . Inset: Transverse section of closely packed microvilli with central filaments. Scale 0.1 μm

Fig. 9. Transverse section of an open rhabdomere (dark-adapted eye). MV microvilli; R retinula cell; RC retinula cell cytoplasm within the rhabdom; RP reflecting pigment cell. Scale 4 μm

Fig. 10. Vesiculated retinula cell cytoplasm and disrupted microvilli at the border of the rhabdom in a light-exposed eye. ER distended endoplasmic reticulum; G Golgi apparatus; MV microvilli; R retinula cell; P retinula cell pigment granule. Scale 1 μm

Fig. 11. Transverse section of a central portion of a rhabdom showing disrupted microvilli of a light-exposed eye. Scale 2 μm . Inset: Phagosome-like bodies (light-exposed eye). Scale 0.5 μm

Fig. 12. Longitudinal section of a reflecting pigment cell. e smooth endoplasmic reticulum; M mitochondria; V vacuole; small arrows reflecting pigment granules; large arrows Golgi apparatus. Scale 1 μm

Fig. 13. Longitudinal section of a retinular cell axon penetrating the basement membrane. Note the abundance of mitochondria in this region. A axon; BM basement membrane; M mitochondria; large arrow basal lamina; small arrows glial cell processes; asterisk microtubules. Scale 1 μm

Fig. 14. Transverse section of an irregularly situated pigment cell. Asterisk carotenoid-like pigment granule. Scale 2 μm

Fig. 15. Longitudinal section in the region of the basement membrane and the eye-capsule. A axon; BM basement membrane (black arrows basal lamina); DP proximal extension of the distal pigment cell; E eye-capsule membrane (white arrow basal lamina); G glial cell; RP reflecting pigment cell. Scale 3 μ m

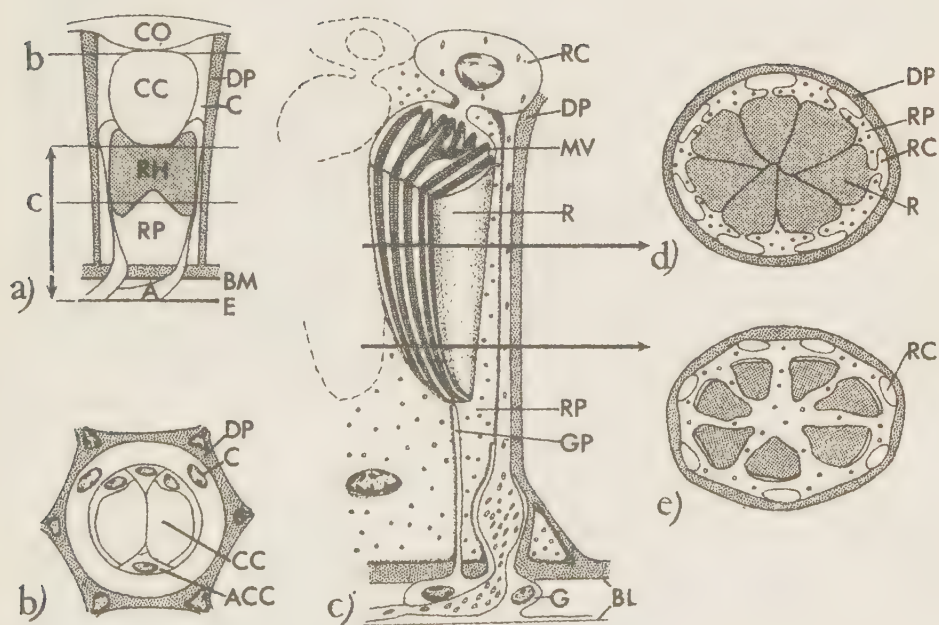
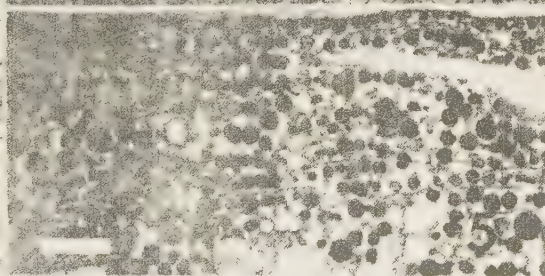
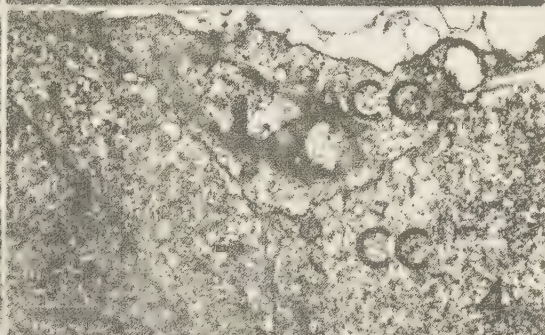
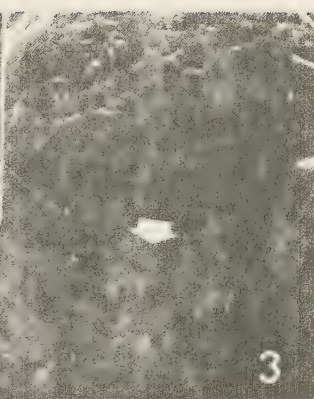
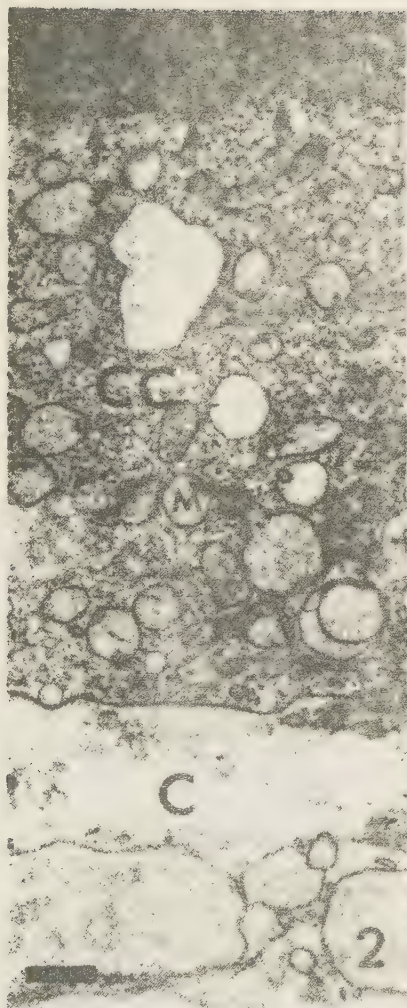
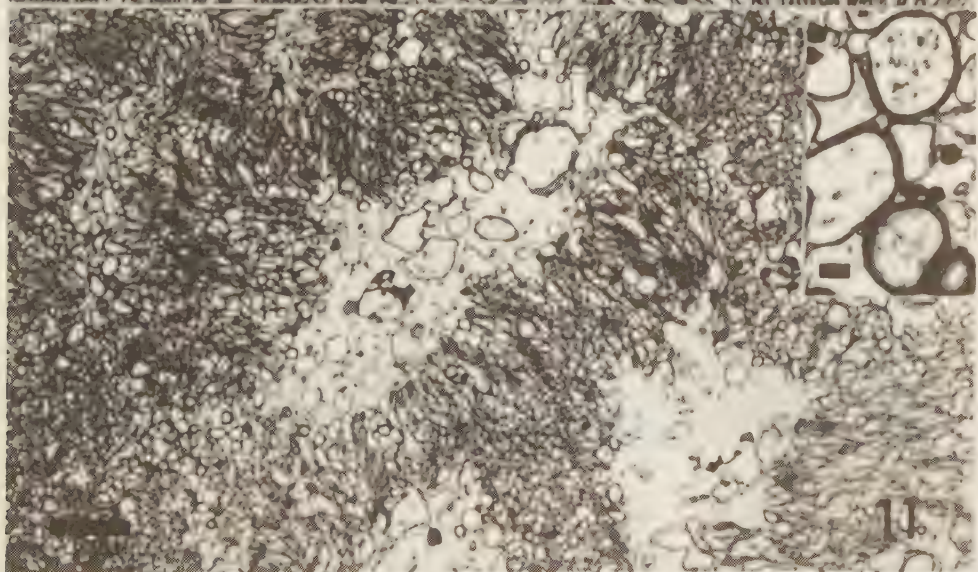
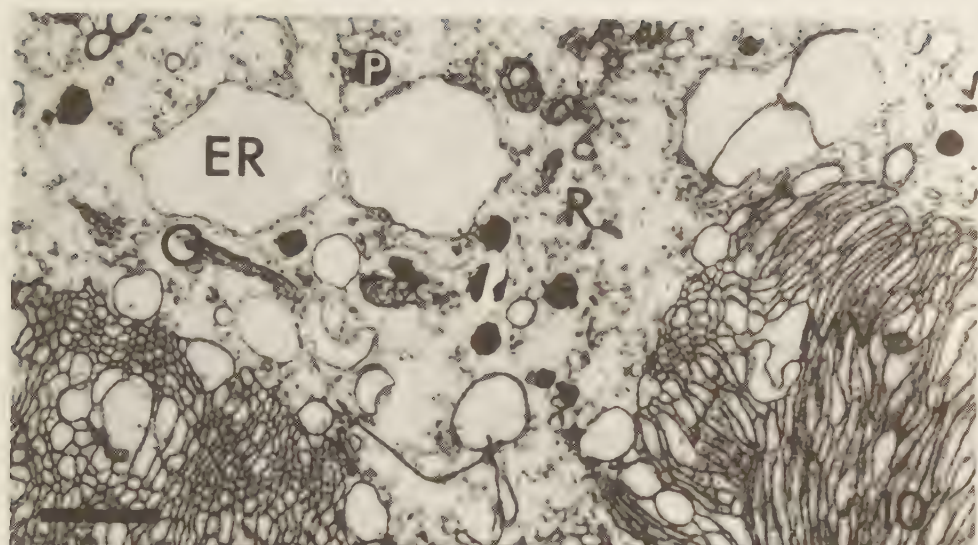
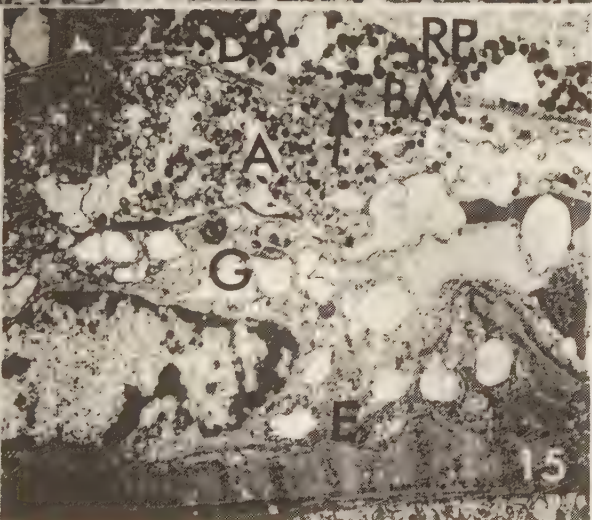
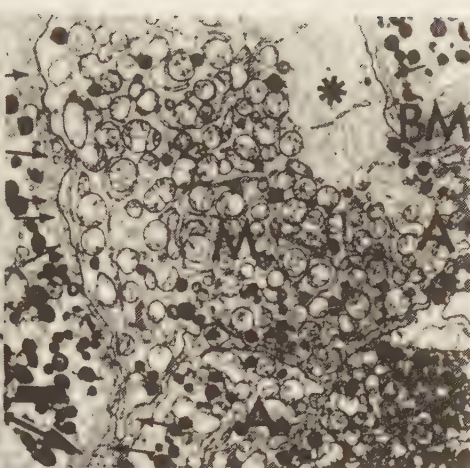
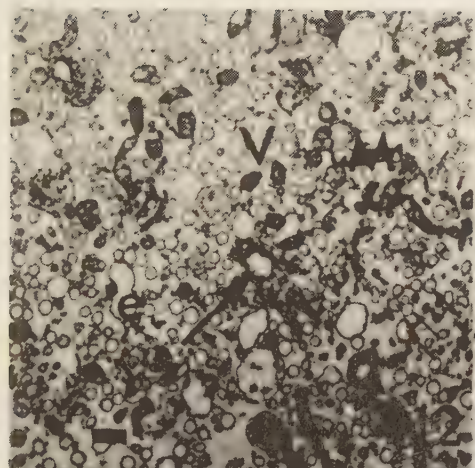


Fig. 1.









The ERG Spectral and Absolute Sensitivities of a Deep-Water
Crustacean (Cirolana borealis)

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Deep-Water Crustacean

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Summary. 1. The electroretinogram (ERG) of the dark-adapted compound eye of Cirolana borealis has a corneal negative on response. The latency period varies between 140 and 8 ms and depends on stimulus intensity. The flicker fusion frequency is 14 Hz.

2. The peak relative spectral sensitivity has a broad shoulder around 495-528 nm, corresponding to a visual pigment of 514 nm, according to Dartnall's nomogram curves. Only one pigment appears to be present.

3. The ERG visual threshold sensitivity, defined as a 5 μ V response at 495 nm, is $2.1 \cdot 10^8 \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ ($n = 17$) and $1.7 \cdot 10^7 \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ ($n = 1$). Only a few quanta of light are needed to elicit a response in the Cirolana eye.

4. The electrophysiological results confirm that the Cirolana eye is well adapted to a dim-light environment and is a typical scotopic eye.

Introduction

Dim-light environments demand special adaptations of visual organs if these are to cope with the low number of available photons. Such adaptations include anatomical, optical and physiological specializations (Snyder 1979; Land 1981). A number of investigations of the eye structure (Doflein 1903; Bursey 1975; Elofsson and Hallberg 1977; Meyer-Rochow and Walsh 1977, 1978; Meyer-Rochow 1978, 1981a; Hallberg et al. 1980; Nilsson 1982) and optics (Land et al. 1979; Andersson and Nilsson 1981; Nilsson and Nilsson 1981) of deep-sea crustaceans have been made. Little, however, is known of the physiological adaptations of these eyes. Electroretinogram (ERG) measurements have been performed on the amphipod Pontoporeia (Donner 1971), and these showed that the eyes have a very high sensitivity. Based on the anatomy and optics of the eye of the isopod Cirolana borealis, a high visual threshold sensitivity was to be expected (Nilsson and Nilsson 1981).

Results of the ERG, flicker fusion frequency, the relative spectral sensitivity and the visual threshold sensitivity of the Cirolana eye are presented in this investigation in which, for the first time, special precautions have been taken to preserve an intact anatomy and not to introduce light-induced damage to the photoreceptor membranes (Loew 1976; Meyer-Rochow 1981a; Nilsson 1982). Our results show that the Cirolana eye is spectrally well-adapted to ambient light, and is also highly sensitive.

Material and Methods

The isopod Cirolana borealis Lilljeborg (suborder: Flabellifera) used in the experiments was obtained in baited traps at about a 100-m depth in Raunefjord, south of Bergen, Norway. The traps were brought to the surface at night (middle of September and March, cloudy sky) while the ship's lanterns were off. The contents of the traps were placed in light-proof tanks and the animals were sorted out in the laboratory expeditiously (less than 10 min) under a red safety light. They were shipped alive by air in Dewar bottles to the Tvärminne Zoological Station in Finland. At the Tvärminne laboratory, where the ERG measurements were made, the animals were transferred to wide aquaria in a light-proof chamber cooled to 3⁰ C. Water from the sampling station was added to a height of about 3 cm to provide for adequate oxygenation without aeration.

All handling of the animals was done either in complete darkness or using infra-red (IR) light and image converters (Find-R-Scope, FJW Industries). The total dark-adaptation time before the experiments started was 4 days. The system for stimulation and recording was the same as used by Lindström (1982). The stimulus light source was an Osram (6V, 15W) microscope lamp powered by a constant-voltage device. Double-interference filters (Schott, half-band width 7-16 nm), ranging from 393 to 673 nm in steps of about 20 nm, could be inserted in the optical path. The intensity of the stimulus was controlled by a circular neutral density wedge and square even log-unit neutral density filters. The light output

was calibrated in absolute units ($\text{quanta} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$, Lindström 1982). The stimulus time was set at 300 or 600 ms.

In the flicker fusion frequency (FFF) experiments, a Philips 9103 stroboscope was used. The light intensity was changed in steps of one log unit using neutral density filters. The absolute energy output of the broad-band flickering light (duration less than 10 ms) (spectral peak at about 600 nm) could not be measured owing to inadequate measuring equipment; but an estimation was made using the highest frequency obtainable with the stroboscope (240 Hz) and by comparing the elicited response amplitude of the Cirolana eye to those evoked by defined intensities in the 600 nm region of the spectrum as measured when determining the spectral sensitivity. With two neutral density filters, the test light evoked c. 100 μV responses and with three such filters about 30 μV (criterion response in the $S(\lambda)$ measurements was 50 μV).

The glass electrodes were drawn with an Ealing microelectrode puller, and the tips were cut off to an outer diameter of about 10 μm . The electrode was painted black with drawing ink about 0.1 mm from the tip to make the electrode visible in IR light. The measuring electrode was filled with a 1 M NaCl solution and was connected to a Tektronix 5031 dual-beam storage oscilloscope. Most recordings were made in the 50 $\mu\text{V}/\text{div}$ setting and the visual threshold measurements were made in the 20 $\mu\text{V}/\text{div}$ setting.

Experimental Procedure

The excised heads were pinned to a piece of cotton immersed in salt water in the experimental chamber. This chamber was

water-cooled to a constant temperature of 10° C during the experiment. The salt water consisted of filtered and UV-irradiated (several hours) sea water from the sampling station. The neutral electrode was inserted in this "Ringer bath" enabling contact with the preparation. During preparation, using IR image converters mounted on a Wild-5 stereomicroscope, each animal was illuminated by IR light (2 Kodak Wratten 87 gelatin filters were inserted in the ray path of white light). A small hole was punched through the corneal cuticula of the eye and the recording electrode was lowered to contact the hole. (Responses cannot be recorded from the surface-intact cornea.) Most recordings were made on central ommatidia, although other eye regions were tested. The preparation time took between 10 and 30 min.

Upon repeated stimuli using maximum intensity of the preparation light, no response was ever recorded from the eyes. Five preparations were done using yellow safety light (with a narrow peak at 590 nm, filtering out all shorter wavelengths). The advantage was that the preparation time became shorter, 5 to 10 min. The preparations maintained good stability, in some cases up to 8 h. The mean experiment lasted about 4 h.

Histology

After the ERG measurements, some of the excised heads were dissected and fixed according to Karnovsky (1965) in a glutaraldehyde/paraformaldehyde mixture (Ca^{2+} omitted) for

3 h at 4⁰ C and postfixed in 2% OsO₄ for 2 h at 4⁰ C. Both fixatives were in 0.2 M cacodylate buffered to pH 7.2. Dehydration was performed in an alcohol series, blockstaining in 0.5% uranyl acetate/1% phosphotungstic acid and embedding in Vestopal W. Sections for electron microscopy were stained in uranyl acetate for 30 min, in lead citrate for 2 min and examined in a Zeiss EM 10 or in a Jeol 100 CX.

Results

Electroretinogram (ERG) measurements were recorded from 55 eyes (low frequency AC amplification). Visual threshold was measured from dark-adapted eyes from 17 animals. All the tested eyes yielded uniform spectral sensitivity curves. The electrode was inserted in central eye regions in most cases. Other locations did not affect the results. Recordings were made in the time interval 8.00 p.m. to 3.00 p.m. No difference in sensitivity caused by diurnal rhythm was found.

The electroretinogram (ERG)

On a dark-adapted eye a light stimulus elicits a monophasic ERG with a steep corneal-negative on response followed by a decay to baseline. A positive off response results at the cessation of the stimulus in most cases (Fig. 1 b, c). Occasionally it is absent. The eye responds to increasing light

intensities with an increase in amplitude. In a majority of preparations, the peak amplitude lies between 300 and 400 μV for a monochromatic flash of 495 nm and with an irradiance of $8.1 \cdot 10^{13} \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$. The highest peak amplitude recorded was 980 μV . At very high stimulus intensities a minute break in the shape of the on response frequently occurs close to peak amplitude (Fig. 1 c). However, at very low intensities the ERG consists of only a slow negative potential declining to baseline (Fig. 1 a).

The response-intensity curve ($V/\log I$) has the characteristic sigmoid shape found in arthropods and vertebrates, and is satisfied by the equation $\frac{U}{U_{\max}} = \frac{I}{I + I_H}$ shown by Naka and Rushton (1966) (U = response at intensity I , $U_{\max}$ = response at saturation point, I = light intensity of stimulus light, I_H = light intensity at the half saturation point). Most preparations give a high threshold sensitivity (see below) and a high peak amplitude, but in some the slope of the $V/\log I$ curve is flat as a result of peak amplitudes of about 100-150 μV , although the threshold sensitivity is maintained (Fig. 2). The latter preparations were generally more easily exhausted during the experiment. These did not last more than a couple of hours compared with a mean of about 4 h. The potentials, irrespective of peak amplitude, show that $V/\log I$ functions increased to saturation over 4 log units. The linear part of the response-intensity curve never extends more than two powers of ten (Fig. 2).

The onset of the ERG is delayed, and at constant temperature it depends on the stimulus intensity (Figs. 1 d, 2). The latency

period varied between 140 and 8 ms, becoming shorter with increasing intensity. Exposures to increasing stimulus intensities, extending about 1.5 log units above visual threshold, caused a fast exponential decrease in latency. Thereafter, the decrease was slow until the response saturation level. Intensities above this level shorten the latency to its minimum value, c. 8 ms.

Flicker Fusion Frequency (FFF)

A preliminary study (of seven animals) of the FFF of the dark-adapted Cirolana eye was done. When exposing the eye to orange-red flickering light, the FFF increased with increasing light intensities (Fig. 1 e). Most animals have an FFF of $14 \text{ Hz} \pm 1.2 \text{ Hz}$ at the same light intensity. One animal, however, showed a 20 Hz flicker, and fusion occurred at less than 24 Hz. (The minute regular 50 Hz pattern seen in the oscilloscope trace, Fig. 3, is an artifact induced by the mains voltage.)

The Relative Spectral Sensitivity $S(\lambda)$

The $S(\lambda)$ measurements were performed using the method of Lindström (1982). A criterion response of $50 \mu\text{V}$ was chosen because this value falls on the beginning of the linear part of the $V/\log I$ curve, thus demanding only moderate or low stimulus intensities (a necessary precaution for this sensitive eye (Nilsson and Lindström, 1982)). During the experiments, the sensitivity level remained stable in most cases; if not, the drift was corrected for.

The $S(\lambda)$ curve obtained had its maximum between 495 and 528 nm (Fig. 3). Compared with the nomogram curves of Dartnall (1953) for vertebrate rhodopsins in low concentrations, the curve should have its maximum at 514 nm. A deflection of the curve is present at about 512 nm, compared to the ideal shape of an absorption curve. The cause of this deflection has not been investigated; plausible explanations are either a minor calibration error in the stimulating system or a filtering, or reflecting, property of the dioptric elements. No difference was found between sensitivity curves of male and female animals. One selective adaptation experiment was performed that showed no tendency to change the shape of the $S(\lambda)$ curve. The ERG has the same shape at all wavelengths.

Visual Threshold Sensitivity

Excised heads were prepared either in IR or yellow safety light and were then left to adapt for 10 min (IR preparations) or 30 min (yellow light). No difference in sensitivity between the two groups was observed. The criterion response amplitude for determining the threshold sensitivity of the Cirolana eye is set at 5 μ V, which is just above the noise level of the system. Only clearly detectable and stable (repeatable) responses have been used (Fig. 1 a). The duration of the test stimuli has been 600 ms, which is long enough to allow the slow response to develop completely. The interstimulus time was set at 2 min. This repetition rate provided the eye time to recover completely between the test flashes and prevented any light adaptation to occur during the

test run, which consisted of about 20-30 flashes (see also Donner 1971). The wavelength of the test stimuli was 495 nm. At the onset of the experiment, the first flash slightly depressed sensitivity and hence only later responses have been used.

The highest sensitivity measured for the eye, at the 5 μV response level, was at a photon flux of $1.7 \cdot 10^7 \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$, but this concerned only one preparation. The mean sensitivity value recorded was $2.1 \cdot 10^8 \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ ($n = 17$). This corresponds to an irradiance of $8.4 \cdot 10^{-9} \mu\text{W} \cdot \text{cm}^{-2}$. Fig. 1 a shows a criterion response. The latency and the rise time were slow compared with responses at higher intensities. The response was well above the noise level of the recording system. Typical of these sensitive dark-adapted eyes is that even short exposures to light of strong irradiance (more than $10^{12} \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$) can destroy the eye by causing morphological changes and a loss of sensitivity (Nilsson and Lindström 1982). Two animals, kept in total darkness, were tested 2 months after capture. They displayed no signs of decreased sensitivity.

If the various factors that influence the number of quanta absorbed by the rhabdom are considered, an estimate of the ultimate sensitivity at the photoreceptor membrane level can be roughly calculated. The light gathering area of the Cirolana ommatidium is about $7800 \mu\text{m}^2$ (the effective lens diameter is c. $100 \mu\text{m}$; Nilsson and Nilsson 1981). During the first 300 ms, the response reaches the criterion response and the ommatidium has received about 5400 quanta (flash time = 600 ms, intensity = $2.1 \cdot 10^8 \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$). Attenuation can be expected to occur at the cornea and crystalline cone. According to Kaplan and Barlow (1975), the cornea of Limulus attenuates the incident light about

one log unit. If this value is used for the Cirolana eye, it means that 540 quanta remain to be absorbed. Nothing is known of the absorbance efficiency of the Cirolana rhabdom or its visual pigment, but some crustacean decapods, e.g. Homarus (Bruno et al. 1977), Procambarus and Orconectes (Goldsmith and Wehner 1977) and Leptograpsus (Stowe 1980) have been found to have about 80-90% efficiency. Using the 90% value, about 480 quanta are left to be considered. The Cirolana ommatidium possesses seven rhabdomeres (Nilsson and Nilsson 1981), thus c. 70 quanta remain to be absorbed by one rhabdomere. Considering the mass response characteristics of the ERG and the limitation set by the noise level of the recording system, the true visual threshold most likely is below this value. It seems justified to predict that only a few quanta are enough to elicit a response.

Histology

The anatomy of the dark-adapted eye of two specimens was studied after the end of the experiments (duration 3½ and 7 h). The cytoplasm and its compartments were fairly well preserved. Some observed changes, compared with untreated dark-adapted eyes (Nilsson 1982), involved the retinula cells, which have 1.) swollen mitochondria with disrupted cristae, 2.) a distended endoplasmic reticulum and 3.) a general vesiculation tendency of the cytoplasmic matrix (Fig. 4). The microvilli of the rhabdom are well preserved, with only small disruptions and swellings (Figs. 5, 6). The cytoplasmic folds bearing the microvilli were narrower in one

preparation (Fig. 5) and normal in one (Fig. 6) when compared with untreated eyes. Finally, retinula cell pigment granules were found to be close to the rhabdom or occasionally located in the cytoplasmic folds. The preparation with the lowest physiological stability and sensitivity showed the gravest cellular changes.

Discussion

The ERG (AC recording) of the dark-adapted compound eye of Cirolana borealis has a fast negative on response, a slow decay to baseline and a positive off effect. These components are typical of the ERG of crustacean apposition eyes (Hanoaka 1950; Ruck and Jahn 1954; Donner 1971; Hertel 1980; Lindström 1982) and in several insects (Autrum 1958; Swihart 1972). At low stimulus intensities the ERG is monophasic. A minute break is present in the on response at high stimulus intensities, perhaps unveiling a second component, making the response diphasic. This would agree with the diphasic ERG (DC recording) found, at high stimulus intensities, in the isopods Armadillidium, Porcellio and Ligia (Benguerrah and Carricaburu 1976). However, in the latter case, steel electrodes were used, and this causes an artifactual second phase according to Kugel (1977). This suggests that the break in the Cirolana ERG must be attributed to some other factor.

The presence of an off effect is considered to be a reflection of a true phenomenon of the eye and the discrepancy in the ERG, i.e. with and without an off effect, is most likely due to different

electrode depths. This was also verified by Donner on the ERG of Pontoporeia affinis (Donner, personal communication).

The FFF of the dark-adapted eye of Cirolana was proved to be 14 Hz, although the responses of the eye of one animal showed a fusion at 24 Hz. Factors such as the light adaptational level and background intensity influence the FFF, and an increased stimulus intensity, for example, yields higher FFF values (Autrum and Stocker 1950). The latter has also been observed in the Cirolana eye. The highest FFF, 24 Hz, cannot, however, be attributed to light adaptational effects, because all the eyes were exposed to the same light intensity and gave an FFF of 14 Hz. It is not known whether the depth of the electrode can influence the FFF, and at present the 14 Hz value is considered to be the relevant one for the Cirolana eye.

The obtained flicker fusion frequency of Cirolana is low compared with terrestrial isopods, e.g. Armadillidium officinalis (24 Hz), Porcellio scaber (32 Hz) and Ligia italica (78 Hz) (Benguerrah and Carricaburu 1976). A higher value, 120 Hz, was obtained in Ligia occidentalis (Ruck and Jahn 1954). A correlation between the number of ommatidia and FFF has been proposed for terrestrial isopods by Benguerrah and Carricaburu (1976), but this does not seem to hold true for the Cirolana eye. It comprises c. 60 ommatidia compared with about 20 in Armadillidium and Porcellio (Nemanic 1975), but still the Cirolana FFF is lower than that of the other two species. The explanation of this may be found upon comparing the FFF of diurnal and nocturnal insects. Both types of insects may have several thousand ommatidia, but the resolving power of diurnal insects often exceeds a hundred cycles per second

(Campan et al. 1965; Miall 1978; Meyer-Rochow 1981b), while the nocturnal insects do not have an FFF exceeding 50 Hz (Campan et al. 1965). This indicates that the Cirolana eye is a typical scotopic eye in which the temporal resolution is low. The 14 Hz value may also be compared to the 10 Hz FFF for the scotopic part of the dark-adapted human eye (Granit 1962).

Spectral Sensitivity

The relative spectral sensitivity curve of Cirolana has a broad shoulder between 495-528 nm, and, according to Dartnall's nomogram curves (1953), this corresponds to a visual pigment with a maximum absorption at 514 nm. It should be emphasized, however, that a certain red shift (20-30 nm) of the sensitivity curve can occur because of screening pigments (Goldsmith and Fernandez 1968; Fernandez 1973; Kong and Goldsmith 1977). Further, the relative spectral sensitivity curve of Cirolana is narrower than the photopigment curve and this can be explained by a selective absorption of the extreme wavelengths by the dioptric elements. Disregarding these reservations, the curve of Cirolana corresponds well to those of several other crustaceans, e.g. Homarus americanus and Limulus polyphemus (Wald 1968), Pontoporeia affinis (Donner 1971), and several gammarid species (Lindström 1982).

No difference in the shape of the ERG is present for different wavelengths, and, according to Wald (1968), this indicates a single pigment system. Because of the increasing monochromatic properties of water with depth (Tyler 1959), there is also no reason to expect more than one pigment for the eye of Cirolana.

No measurements of the spectral distribution of light at the sampling station were carried out. Using Jerlov's data on oceanic water, type III (1976), an extrapolation down to 60 metres depth was possible and a crude representation of the spectral distribution could be drawn. The spectral sensitivity of the Cirolana eye has been compared with this curve and the correlation is good (Fig. 7).

Visual Threshold Sensitivity

The Cirolana eye responds to a mean intensity of $2.1 \cdot 10^8 \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$, measured at peak wavelength, 495 nm. One preparation exceeded this sensitivity by about one log unit. A comparison of ERG visual thresholds, defined as total quantal flux per cm^2 and second (or $\mu\text{W} \cdot \text{cm}^{-2}$), incident on the cornea of some deep- and shallow-water-living crustaceans and some nocturnal arthropods is seen in Table 1. Man is included for a comparison. This shows that the Cirolana eye fits very well into the included group of highly sensitive arthropod eyes. Compared with other arthropod eyes in the table, the threshold sensitivity of the Cirolana eye is about 1 to 2 log units higher. A further increase in sensitivity by 2 log units is inferred by the difference between ERG-recordings (Donner 1971) and behaviour in the amphipod Pontoporeia (Donner and Lindström 1980). The large light-gathering capacity of the Cirolana eye is optically accomplished by a large numerical aperture (1.0) and by wide acceptance angles between the rhabdomeres (c. 20°), which cause a visual overlap (Nilsson and Nilsson 1981). This, together with anatomical specializations such as hypertrophied rhabdoms and the presence of a reflecting pigment

cell layer, account for the high sensitivity (Nilsson and Nilsson 1981; Nilsson 1982). Visual acuity, however, is sacrificed, as this is incompatible with a large receptor spacing and few receptive channels (Snyder 1977). The Cirolana eye is adapted to see extended sources rather than point sources judged from optical considerations and based on the small pupil size (Kirschfeld 1974).

To be able to compare the tabulated values with other published ones, some general methodological aspects must be considered. Considering the capacity of the equipment, which does not allow accurate measurements below the defined $5 \mu V$ response, a lower visual threshold than that obtained can be expected for the Cirolana eye. A direct comparison between mass recordings (ERG) and intracellular recordings of absolute sensitivity cannot be done. The ERG responses add all signals from the photoreceptors, and single cell responses from individual rhabdomeres can only roughly be calculated. Intracellular sensitivity determinations near the absolute visual threshold, taking advantage of the possibility to recognize and to statistically treat quantal "bumps" (Fuortes and O'Bryan 1972) and to relate these to sensitivity, are in this respect a superior method. The ERG, on the other hand, is a simple and fast method for obtaining a reliable value of the overall threshold sensitivity to incident light on the eye.

If the eye is uniformly illuminated and the exact number of photoreceptors of the rhabdom is known, the ERG can still be used to calculate roughly single cell sensitivity. The Cirolana eye is considered adequately illuminated to perform this calculation. We found that a few quanta of light may elicit a response

in a rhabdomere. This accords with calculations from the crustacean amphipod Pontoporeia (Donner 1971), the spider Lycosa (DeVoe et al. 1969) and the scorpion Androctonus (Fleissner 1977a). Intracellular measurements of single cells of a variety of arthropods also confirm that a few quanta or even one quantum is enough to elicit a response (Fuortes and Yeandle 1964; Scholes 1964; Kirschfeld 1966; Lillywhite 1977; Laughlin et al. 1980).

A good agreement between sensitivity and light intensity of the habitat of Cirolana could occur, although no values of the irradiance at the sampling station were obtainable. A rough extrapolated value can be obtained by using the Jerlov classification (1976) of oceanic water, type III. At a depth of 100 m (sampling station), 0.001% of the surface irradiance (500 nm) remains left. Assuming the surface irradiance (400-700 nm) to be about $10^{17} \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$, at noon on a sunny day in the summer and $10^{14} \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ in the autumn, at Norwegian northern latitudes (Thronsdén and Heimdal 1976), 10^{12} and $10^9 \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$, respectively, are left at this depth. This shows that the Cirolana eye can cope very well with its light environment. It is not known whether single or a few quanta are able to evoke visual perception and behaviour in Cirolana. Such a possibility cannot be ruled out, because Donner and Lindström (1980) have shown that biorhythmical vertical migration can be triggered by only a few quanta of light in Pontoporeia affinis. Very dim light, 2-4 log units above the visual threshold, also entrains the circadian rhythm in the scorpion Androctonus (Fleissner 1977b, c). Our observations (Nilsson) show that the animals are primarily trapped at night.

This is inferred from the fact that when the baited traps are lowered to the sea floor in the evening, the highest number of animals is brought up the next morning. This nightly capture is also verified by results from 24-hour trawls (with an epibenthic sledge) which show that most animals are trapped at night (Brattegard, personal communication). Both of these observations stress the fact that dim light is of biological importance to Cirolana.

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Figure legends

Fig. 1. Electroretinograms (ERG) recorded from the dark-adapted compound eye of Cirolana borealis. (The curves in a, b, and c are from different preparations. Flash duration in a-c = 600 ms and in d = 300 ms.) a. A 5 μ V criterion response used for the visual threshold measurements. b. A 50 μ V criterion response used for the relative spectral sensitivity measurements (the spike-like response is a disturbance caused by movements of the antenna). c. A response to a bright light stimulus. d. The relationship between latency time and amplitude of the response at constant temperature (see also Fig. 2) shows that an increase in amplitude is followed by a decrease in latency time. (Latency time = the interval between the onset of the light stimulus and the beginning of the on response.) e. Responses to an increased stimulus rate of flickering light (periodicity in cycles per second, Hz). Fusion occurs at around 14 Hz.

Fig. 2. Response/log intensity curves (μ V/log I) and latency time/log intensity curves for the dark-adapted compound eye of Cirolana borealis. A and B = μ V/log I curves for two eyes with different response saturation levels (λ = 495 nm). C and D = the dependence of latency time on stimulus intensity for two different preparations. I = log relative intensity.

Fig. 3. The log relative spectral sensitivity curve of the dark-adapted Cirolana eye ($n = 14$) (solid line). Stippled line: the Dartnall's nomogram curve for a visual pigment with an absorption maximum at $\lambda = 514$ nm. Error bars represent standard error of the mean. Abscissa: the wavelength, λ (nm), plotted upon a linear scale of frequency. Ordinate: log relative sensitivity ($\log S$).

Fig. 4. The fine structure of a retinula cell in the rhabdom region of the compound eye of Cirolana borealis. Fixation was done immediately after 4 h of electroretinograph measurements (preparation: excised head). G = Golgi complex, white arrows = distended smooth endoplasmic reticulum, small arrows = disrupted mitochondrial cristae, asterisk = cytoplasmic vesicle. Scale = 1 μ m. 8250X

Fig. 5. The fine structure of the rhabdom in the compound eye of Cirolana borealis. Fixation was done immediately after 4 h of electroretinograph measurements (preparation: excised head). Note well-organized microvilli. Mv = microvilli, arrow = narrow cytoplasmic sheets between the microvillar lamellae of the rhabdom. Scale = 2 μ m. 5280X

Fig. 6. The fine structure of the rhabdom of the compound eye of Cirolana borealis. Fixation was done immediately after 7 ½ h of electroretinograph measurements (preparation: excised head). Note wider space (compare with Fig. 5) between the microvillar lamellae of the rhabdom. Mv = microvilli, Rc = retinula-cell fold between the microvillar lamellae of the rhabdom, Rp = reflecting pigment cell, arrow = distended and disrupted microvilli. Scale = 1 μ m. 6180X

Fig. 7. Spectral distribution of light, downward irradiance (relative transmission, in %), at a depth of 60 m (broken line), for oceanic water type III (freely after Jerlov (1976)). This is compared with the relative spectral sensitivity curve of the compound eye of Cirolana borealis (solid line). Arrow = the theoretical curve of a visual pigment with a maximum at λ = 514 nm (constructed from the Dartnall's nomogram curves (1953)).

Table 1. Electroretinogram visual thresholds, measured at peak wavelength (maximum sensitivity), of some arthropods. Psychophysical measurements in man are included for comparison. (Where other units have been used in the cited works, these have been converted to the units in the table.) Values within brackets, for the Cirolana eye, are for the highest recorded sensitivity ($n = 1$), and the other values are means from 17 specimens.

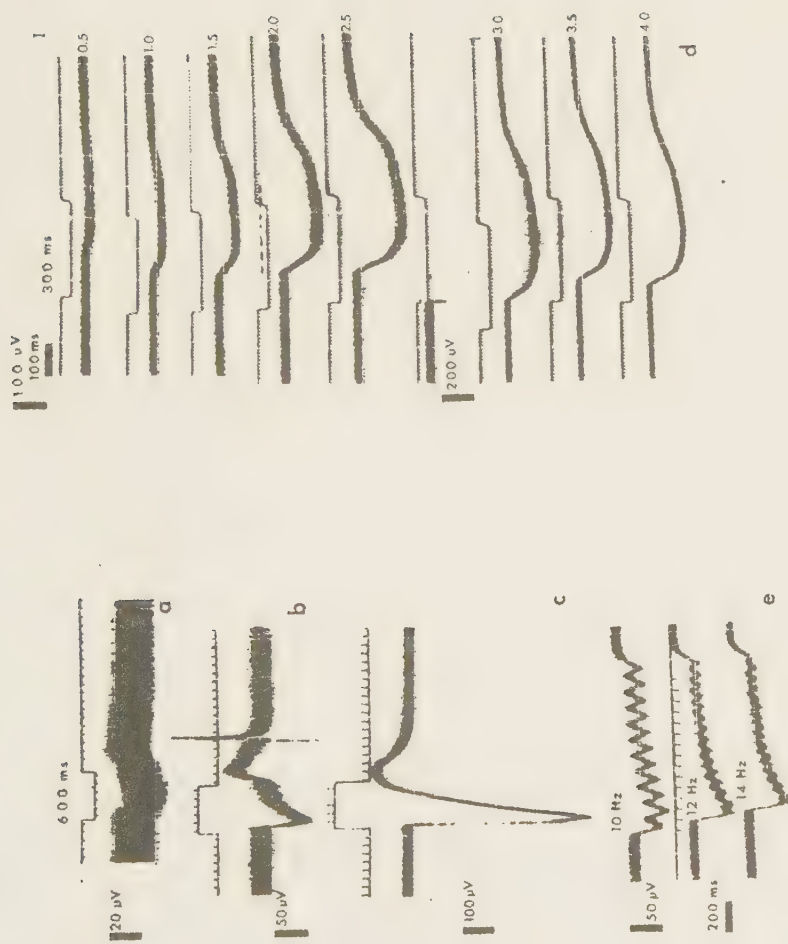


Fig.1.

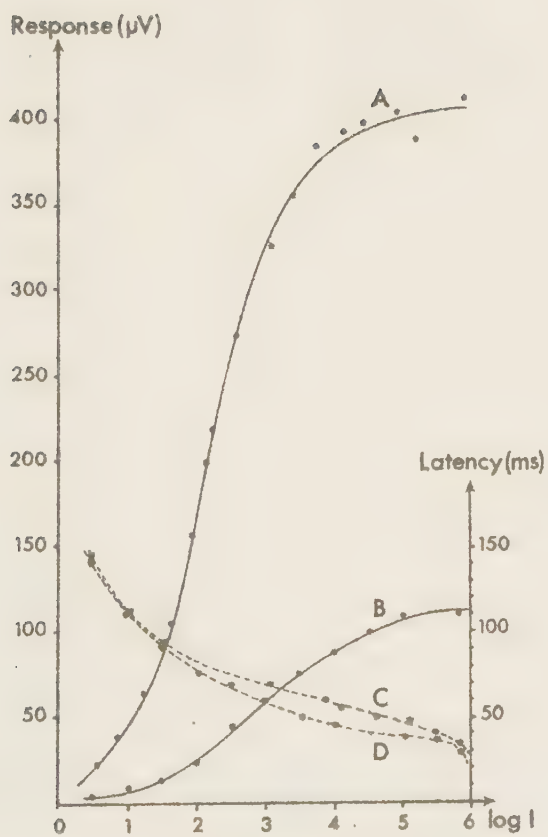


Fig. 2.

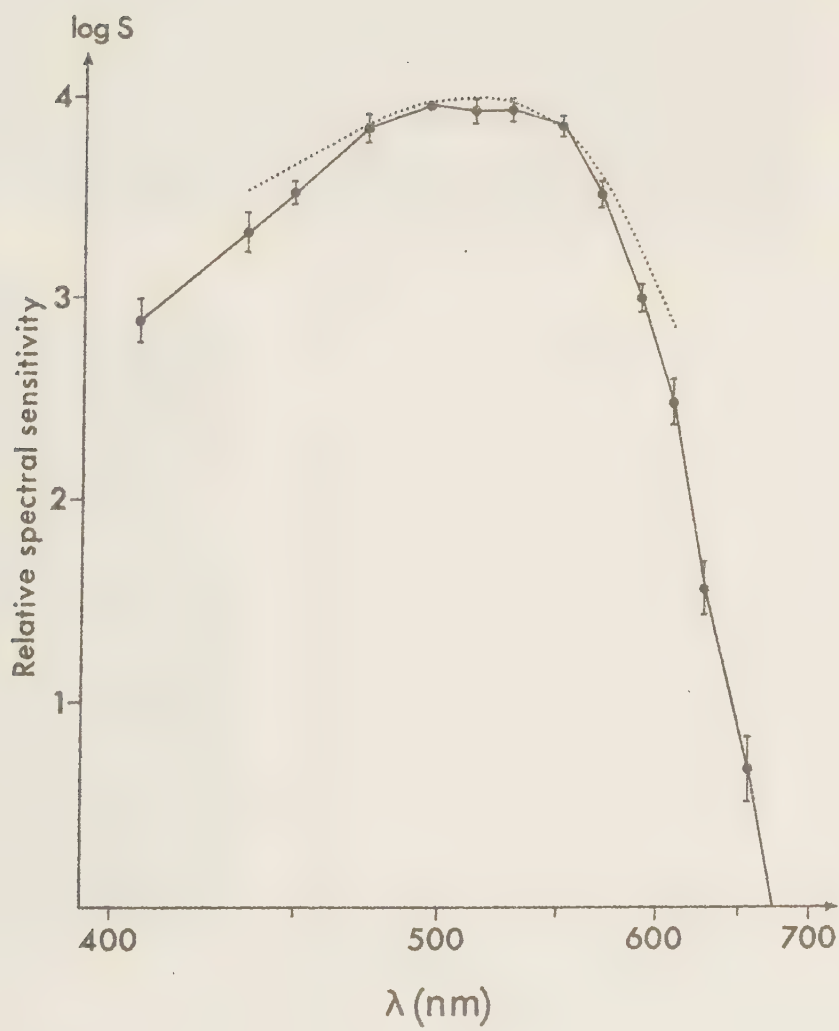


Fig. 3.

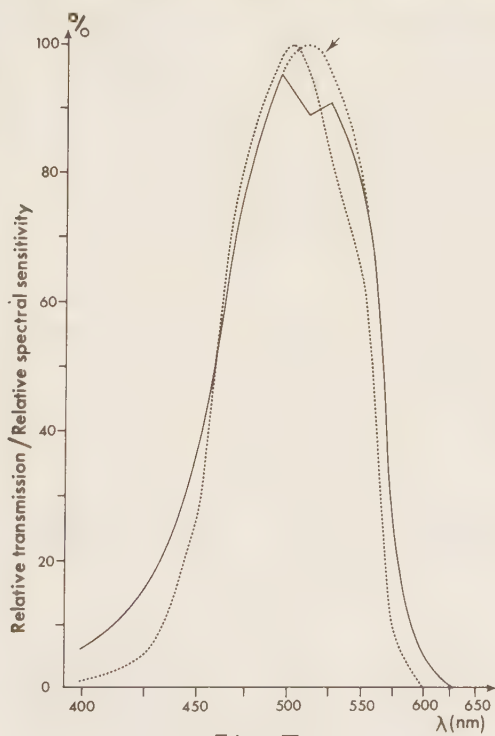
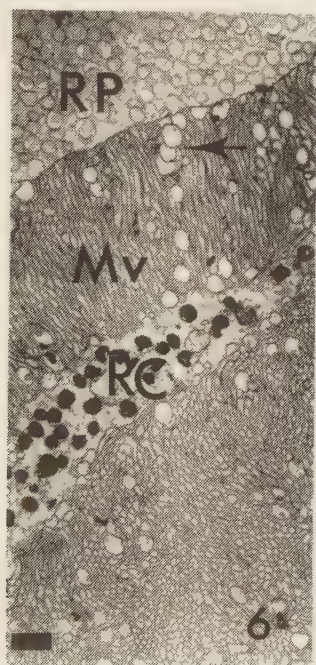
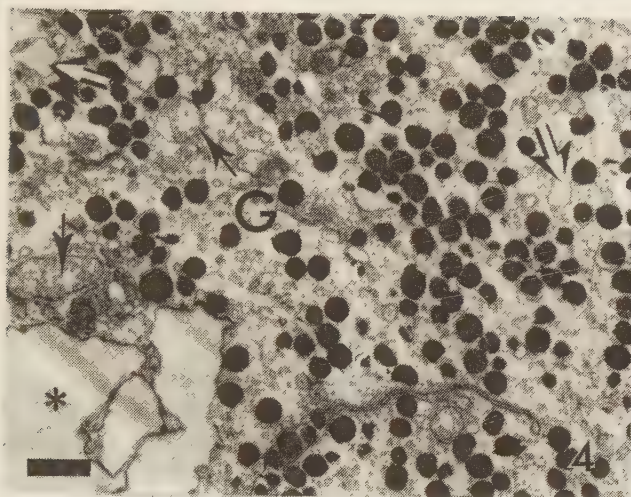


Fig 7

Table 1. Visual ERG-thresholds of some arthropods

Species	λ nm	criterion response (μV)	Sensitivity $qu \cdot cm^{-2} \cdot s^{-1}$	$\mu W \cdot cm^{-2}$	Reference
Crustacea					
<u>Cirolana borealis</u>	495	5	$2.1 \cdot 10^8$ ($1.6 \cdot 10^7$)	$8.4 \cdot 10^{-9}$ ($6.4 \cdot 10^{-10}$)	This work
" "	495	50	$1.1 \cdot 10^9$	$4.4 \cdot 10^{-8}$	" "
<u>Pontoporeia affinis</u>	533	10	$1 \cdot 10^8$	$4 \cdot 10^{-9}$	Donner 1971
<u>Homarus americanus</u>	525	50	$2.5 \cdot 10^{10}$	$9.5 \cdot 10^{-7}$	Wald 1968
<u>Procambarus clarkii</u>	550	50	$7.2 \cdot 10^9$	$2.6 \cdot 10^{-7}$	" "
<u>Libinia emarginata</u>	490	50	$4.0 \cdot 10^{10}$	$1.6 \cdot 10^{-6}$	" "
<u>Carcinides maenas</u> (510)		50	$1.3 \cdot 10^{11}$	$5.1 \cdot 10^{-6}$	" "
Merostomata					
<u>Limulus polyphemus</u>	520	50	$2.1 \cdot 10^9$	$8.0 \cdot 10^{-8}$	Wald 1968
Arachnoidea					
<u>Androctonus australis</u>	493	1	$1.2 \cdot 10^9$	$4.7 \cdot 10^{-8}$	Fleissner 1977a
<u>Lycosa miami</u>	505	50	$2.6 \cdot 10^{10}$	$1.0 \cdot 10^{-6}$	DeVoe 1969
Man	510	Psych. Ph. test	$5.1 \cdot 10^4$	$2.0 \cdot 10^{-12}$	Hecht et al. 1942 Wald et al. 1963

RETINAL DAMAGE AND SENSITIVITY LOSS OF A LIGHT-SENSITIVE
CRUSTACEAN COMPOUND EYE (CIROLANA BOREALIS):
ELECTRON MICROSCOPY AND ELECTROPHYSIOLOGY

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Running title: Retinal damage in Cirolana borealis (Crustacea:
Isopoda)

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SUMMARY

1. The compound eye of the deep-water-living crustacean Cirolana borealis has been exposed to quantified white light exposures and analyzed by electron microscopy and electrophysiology (ERG) and the results have been correlated.

2. The threshold for damage lies between 117 lux ($0.47 \text{ W}\cdot\text{m}^{-2}$) and 1250 Lux ($4.9 \text{ W}\cdot\text{m}^{-2}$). Severe structural derangement of the retinula cells is caused by daylight exposures of more than $70 \text{ W}\cdot\text{m}^{-2}$, whereupon the amplitude of the electroretinogram (ERG) is abolished.

3. No recovery of the retinula cell organization or normalization of the ERG occurs after daylight exposure and a dark period of up to 5 days. The induced retinal damage is "irreversible". Thus, the light exposure triggers the breakdown of the retinula cells, which continues in darkness.

4. A novel type of photoreceptor membrane shedding is described for both dark-adapted and light-exposed eyes.

5. The microvilli of the rhabdom are closely aligned by "tight junction"-like connections and the corresponding extracellular spaces are maximally limited.

6. Hence, morphologically and functionally, the Cirolana eye is strictly adapted to a dim-light environment and is destroyed by too intense illumination.

INTRODUCTION

Recent investigations (see below) have engendered new interest in the morphology and function of dim-light-adapted compound eyes (scotopic eyes). Optically, these eyes have lenses with large acceptance angles (Land, 1981; Nilsson & Nilsson, 1981; Stavenga, 1979) and visual overlap (Nilsson & Nilsson, 1981). Both eye-bearing deep-water-living crustaceans (for review, see Hallberg, 1978) and other invertebrates living in environments with low light intensities have developed reflecting layers, tapeta (Land, 1972, 1981; Miller, 1979). This is considered to be an adaptation to ensure an efficient photon capture (Snyder, 1977; Snyder et al., 1977).

Further, in some deep-sea crustaceans the area of the photoreceptor membranes is enlarged, and thus the rhabdoms appear as hypertrophied (Elofsson & Hallberg, 1977; Hallberg et al., 1980; Meyer-Rochow, 1981; Nilsson, 1982). These morphological adaptations have been demonstrated in crustaceans to be associated with a high visual threshold (Donner, 1971; Lindström & Nilsson, 1982). Functionally, however, the deep-sea-adapted eyes have been shown to be vulnerable to excessive light. Even moderate amounts of natural daylight or artificial light cause the photoreceptor membranes to break down (Loew, 1976; Meyer-Rochow, 1981; Nilsson, 1982; Tuurala & Lehtinen, 1971). A similar photo-induced destruction was also observed in Limulus (Behrens & Krebs, 1976), in visual mutants of Drosophila (Cosens & Perry, 1972; Harris & Stark, 1977), and Procambarus (Kong & Goldsmith, 1977), and in a number of vertebrates (for references, see Williams & Baker, 1980).

A correlation between the morphology of the retina and the sensitivity of the eye has been established in the vertebrate eye (Kuwabara, 1970; Lawwill, 1973a, b; Noell et al., 1966). The present investigation was undertaken to determine the effects of quantified light exposures to the eye of the deep-water-living crustacean Cirolana borealis and to correlate eye morphology and sensitivity. The results show that a correlation exists and that normal eye function is abolished by too strong illumination.

MATERIALS AND METHODS

Cirolana borealis Lilljeborg (Crustacea: Isopoda) were obtained at night in baited traps from c. 100-m depth in Raunefjord, south of bergen, Norway. All regular lights were turned off on board the ship. The animals were sorted in less than 10 min in red safety light and subsequently shipped by air to the Tvärminne Zoological Station, Finland, where the histological fixations and the electrophysiology were performed. The well-fed animals were transferred to 500 ml glass beakers (Jena), filled with c. 3 cm of water from the sampling station, and with two or three animals in each beaker. These were kept in a 3°C cooling chamber in a wide light-tight box in a co-ordinate system, allowing for easy identification of the beakers in the dark. All handling of the animals took place in the dark using infrared (IR) light and

IR image converters (Find-R-Scope, FJW Industries). The animals were dark-adapted 1-7 days prior to the experiments (exp. 1), and during the experiments the animals were exposed to the light intensities, presented in Table 1.

After light exposure one batch of animals ($\underline{n} = 50$) was used for histological fixation of the eyes, for light and electron microscopy; and another batch ($\underline{n} = 54$), for electrophysiological measurements (Electroretinogram, ERG).

Additional comments to the experiments:

Exp. 2 ($0.47 \text{ W} \cdot \text{m}^{-2}$): The beaker containing the animals (10°C) was exposed to ordinary room light (illuminance 117 lux). The light was calibrated in terms of $\mu\text{W}/\text{cm}^2$ by using the interference filters from the ERG measurements (see below) and a UVM-8LX Luxmeter calibrated in absolute units by Airam laboratories for a wavelength of 564 nm (Donner & Lindström, 1980). Knowing the filter parameters, an integration of the flux between 393 and 673 nm and in steps of about 20 nm could be done (Fig. 1).

Exps. 3, 4 (4.9 and $9.8 \text{ W} \cdot \text{m}^{-2}$): These exposures took place in a cooling chamber at 3°C . The beaker containing the animals stood on a piece of white styrox and was centred under a vertically mounted Osram 6V 15W (70152) microscope lamp with housing. The diameter of the white light patch was adjusted to give uniform light over the bottom of the beaker and with illuminances of 1250 (exp. 3) and 2500 lux (exp. 4) at the level of the Cirolana eyes, measured with a standard Luxmeter (Dr Bruno Lange, Berlin). The exposure light was calibrated as in exp. no. 1 (Fig. 1).

Exp. 5. Three daylight exposures with different intensities were performed: (1) Sunny day with clouds, for 4 h, with an energy content of $1.4 \cdot 10^6 \text{ J} \cdot \text{m}^{-2}$ ($97 \text{ W} \cdot \text{m}^{-2}$). (2) Sunny day without clouds, for 10 min, with an energy content of $4.2 \cdot 10^4 \text{ J} \cdot \text{m}^{-2}$ ($70 \text{ W} \cdot \text{m}^{-2}$). (3) Sunny day without clouds, for 2 h with an energy content of $9.8 \cdot 10^5 \text{ J} \cdot \text{m}^{-2}$ ($136 \text{ W} \cdot \text{m}^{-2}$).

The beaker containing the animals was kept at less than 10°C in an ice bath outdoors. No shading of the beaker was allowed. The energy content of the exposures was measured with a solarimeter (Kipp and Zonen Solarimeter integrater CC1) positioned next to the beakers.

Histological treatment

Dark-adapted eyes were dissected in the fixative (IR light) and light-exposed eyes, in red safety light (exps. 2-5, Table 1). A mixture of paraformaldehyde/glutaraldehyde according to Karnovsky (1965) with Ca^{2+} omitted was used, fixation time was 3 h. Post-fixation was carried out in 2% OsO_4 for 2 h at 4°C , and 0.2 M cacodylate buffer, pH 7.2, was used throughout. Dehydration was performed in an alcohol series; blockstaining, in 0.5% uranyl acetate/1% phosphotungstic acid; and embedding, in Vestopal W, which was polymerized at 60°C for 48 h. Semithin sections ($1 \mu\text{m}$) for light microscopy were stained according to Richardson et al. (1960), and ultrathin sections for electron microscopy were stained in 1% uranyl acetate (in alcohol) and in

1% lead citrate. The sections were examined in a Zeiss EM 10 and a Jeol 100 CX electron microscopes equipped with goniometers.

Electroretinogram (ERG) measurements

All handling of the animals between light exposure and the first test flash was done in the dark using IR image converters (Lindström & Nilsson, 1982). Each excised Cirolana head was pinned to a piece of cotton, immersed in sea water from the sampling station and placed in the experimental chamber. The preparations were cooled to about 10°C and the temperature was continuously recorded with a thermocouple.

Flashes (duration 600 ms) of monochromatic light ($HW_{max} \leq 10$ nm), focused on the eye, evoked eye potentials, which were recorded through a glass micropipette (tip diameter 10 μ m and filled with 1 M NaCl) on a dual-beam oscilloscope. A readily detectable and repeatable on-response of 5 μ V at 495 nm was used as criterion response in the sensitivity threshold experiments (see also Lindström & Nilsson, 1982). The intensity of the stimulus was adjusted using neutral density filters and a circular neutral density wedge. All recordings were in AC. The stimulus and recording system is described in detail by Lindström (1982). The preparations were allowed to accommodate approx. 30 min before the first stimulus light was presented.

RESULTS

Morphology

Dark-adapted eye (DA)

A starting point for the investigation is the anatomy of the dark-adapted (DA) eye of Cirolana borealis. The eye comprises c. 60 ommatidia, and the dioptric elements of the ommatidium are a corneal lens, which is slightly convex externally and strongly so internally, and a round crystalline cone. Distal pigment cells screen neighbouring ommatidia. The rhabdom is formed by the rhabdomeres of seven retinula cells. Moreover, the rhabdom is fused distally and is open proximally (Fig. 2). Each rhabdomere consists of several cytoplasmic folds originating from the retinular cells and projecting into the rhabdom space. The folds are often multiforked and covered with microvilli (Figs. 2, 3). Neighbouring retinular cells are in close contact and can even share a fold. The microvilli are tightly packed and orderly arranged perpendicular to the light. The retinula cell cytoplasm is confined to thin sheets around the rhabdom and the retinula cell screening pigment granules occur outside the reflecting pigment cell layer, which wedges in between the retinular cells and separates the rhabdom from the rest of the retinular cell (Fig. 3). A more detailed description of the morphology of the Cirolana eye, dark-adapted and daylight-exposed, is found in Nilsson (1982).

Attention is focused below on some details of the structure of the dark-adapted retinula cells (nuclear region), which have a bearing on the results of different light exposures to the eye.

The microvilli of the rhabdom are straightly aligned and 2.5-3 μm long and 60-100 nm wide. Each villus originates separately from a short and thin neck (length and diam. about 25 nm) (Fig. 4). This arrangement may be observed as closed microvillar bases, which is due to a lack in perfect alignment of the section along the necks of the microvilli. A close packing along the circumference of the microvilli appears as an almost "tight junction"-like structure (Fig. 5), which restricts the extracellular space to thin channels (less than 4-6 nm wide) in between the corners of the "hexagonally" packed microvilli (Fig. 6). A slight swelling of the extracellular space is present only in the basal and apical regions of the microvilli (Fig. 4). The distance across aligned microvillar membranes is c. 17.0 nm. The resulting five layers, three electron-dense and two electron-lucent, are equally thick, c. 3.4 nm. No gap between the layers was detected by goniometer tilt.

An electron-dense extracellular deposit is present at each microvillar tip (Fig. 3, inset). The cytoplasmic side of the microvillar membrane is coated with a flocky electron-dense material, and axial filaments occur in each villus. The latter seem to be attached to the cell membrane (Fig. 6).

Large (150-250 nm in diam.) and small (25-60 nm in diam.) vesicles occur in moderate or small numbers in the cytoplasm just beneath the microvilli. Depending on the plane of section, these can be seen connected to the microvilli. These vesicles have a double membrane and their abundance and formation will be dealt with further in the light-exposed eyes (Fig. 4). Occasionally, small (c. 20-30 nm) coated vesicles are found (Fig. 4).

The Golgi complexes are mostly found in the soma region and they are fairly frequent. Small (diam. c. 40-50 nm) and large (diam. c. 0.5-0.6 μ m) single-membraned vesicles are present in the vicinity of the Golgi complex (Fig. 7), and on rare occasions a continuity between the large vesicles and the Golgi cisternae is seen. Small fragments of rough endoplasmic reticulum (RER) are dispersed in the retinula cell cytoplasm. The smooth endoplasmic reticulum (SER) is thin and consists of a large number of membrane-delimited sacs and tubules (Fig. 7). Only a few free ribosomes are present in the cytoplasm. No lysosome-like bodies are found. A number of irregularly shaped vesicles are present in the cytoplasm and these are interpreted as distended cisternae of the smooth endoplasmic reticulum (Fig. 7). The mitochondria are well preserved.

Light-exposed eye (LE)

Eyes were fixed immediately after exposures to different intensities of white light according to Table 1. The anatomy of the retinula cells is altered compared with the totally dark-adapted eye (DA). The changes referred to mostly involve the microvilli, and some of the organelles, such as the Golgi complex, endoplasmic reticulum, mitochondria and pigment granules. Rarely, lysosome-like bodies are observed. Unless otherwise stated, the described differences are the same for different exposure times at the same light intensities.

117 lux (exp. 2): The radial arrangement of the folds bearing the microvillae is only slightly distorted. The microvilli maintain a close packing and an orderly pattern, although they are uneven in thickness (20-200 nm) and are shorter (1-2 μm) than those of the DA eye (Fig. 8). Occasionally, the microvilli are disrupted but then the disruptions are restricted to small areas.

In contrast to the DA eye, a large number of various sized double-membraned vesicles are present at the microvillar bases. Some of these are continuous with the microvilli and some are free in the cytoplasm (Fig. 8). They are also characterized by a fuzzy coat on both sides of the membrane (Fig. 10).

Other changes caused by light are also seen. Small ($\approx 0.1 \mu\text{m}$) and irregularly shaped, free, single-membraned vesicles in the cytoplasm and the Golgi complexes have increased both in size and number. The latter display changes, expressed as fragmentation and hypertrophy, which result in a number of small vesicles intermingled with

larger ones (diam. about 700 nm) (Fig. 12). A number of retinula cell pigment granules have migrated into the rhabdom area and spread into the cytoplasmic folds. They are concentrated half way to the centre of the rhabdom (Fig. 8). Few onmatidia, within the same eye, have less well-preserved microvilli, and these contain a larger amount of pigment granules among the microvillar lamellae, and this is especially the case around the distal rhabdom tip.

1250 lux (exp. 3): No fixations were made immediately after the light exposure.

2500 lux (exp. 4): Light microscopically, the changes to an increased stimulus intensity are seen as a larger disorder of the microvillar lamellae. The fine structure of the microvilli, however, has not radically changed when compared with exp. no. 2. The number of vesicles at the microvillar bases is fairly high (Fig. 9). Except towards an increased number of Golgi complexes, no further changes are observed in the retinula cell cytoplasm, and this also includes the pigment position.

Daylight (exp. 5): An increased distortion of the folds bearing lamellae and the microvilli is caused by daylight exposures (Fig. 13). The packing of the microvilli is loose and the number of local swellings has increased and frequently disrupted microvilli are present (Fig. 15). Only occasionally, a fairly regular pattern of short microvilli (length 1-1.5 μm) is preserved. Frequently, membrane whorls are seen at the microvillar bases (Fig. 11). No major differences have been seen between short and long exposure times at the same light

intensities, whereas a more intense exposure, although of shorter duration, caused more severe morphological changes.

Changes in the retinula cell cytoplasm involving a generally less well-preserved cellular matrix and disrupted mitochondria were frequently found. Pigment granules were found throughout the rhabdom (Fig. 15).

Recovery experiments (exp. 6)

Some animals that were exposed to light according to Table 1 (exps. 2-5) were post dark-adapted to determine if the morphology could be returned to the DA condition.

117 lux: The morphology of the rhabdoms after 12 h of dark adaptation is characterized by regularly organized folds bearing microvilli and by slightly distended microvilli. Thus, a better preservation than fixation immediately after light exposure is present (Fig. 16). Diminished vesiculation at the microvillar bases, similar to the case in the DA eyes, was also found (Fig. 16). The microvilli are about 2 μ m long.

No difference in the general appearance of the cell cytoplasm was observed after recovery as compared with fixation after light exposure.

The pigment granules have migrated from the deep location within the rhabdom and are now situated in the peripheral margins of the cytoplasm, which surrounds the rhabdom. (Rarely pigment granules may be seen in the rhabdom (Fig. 16).)

1250 lux: A 10-min exposure of light followed by 12 and 24 h of dark adaptation elicited a poor rhabdom microvillar morphology, and the situation was still worse after the longer DA period. The microvilli are distorted, manifest dilatations and frequently have collapsed causing the rhabdom to appear vesiculated (Figs. 14, 17).

The cytoplasm of the retinula cells is vesiculated (distended SER), but otherwise the organelles are well preserved. The pigment granules of the retinula cells occur throughout the rhabdom (Fig. 17).

2500 lux: The longer the DA time the worse was the morphology displayed by the microvilli of the rhabdom. Two hours of DA produced the same anatomy as is described for an initial exposure to this light intensity, but 12 h of DA elicited an increased number of membrane-whorls and disruptions. An increased vesiculation was present in the cytoplasm of the retinula cells, but mitochondria were well preserved. Pigment granules were found throughout the rhabdom space, similar to the situation in Fig. 17.

Daylight: Two and five days of DA after 4 h of daylight exposure resulted in a retinula cell anatomy that was severely deranged. The microvilli of the rhabdom were disrupted and membrane residues and vesicles were found throughout (Fig. 18). The cytoplasm was vesiculated and mitochondria were disrupted (Fig. 19). Pigment granules are present among the residues of the rhabdom (Fig. 18).

Electrophysiology -- the electroretinogram (ERG)

Dark-adapted eye (DA)

To study the electrophysiological changes evoked by light exposures to the dark-adapted (DA) Cirolana eye, the electroretinogram (ERG) has been used (Lindström & Nilsson, 1982) for a comparison, and reference is made to the visual threshold sensitivity, the response log intensity function ($\mu\text{V}/\log I$) and to the ERG curve.

The ERG of the DA eye has on exposure to a light stimulus, a cornea negative on-response with peak amplitudes between 300 and 400 μV at the highest stimulus intensities. The response slowly decays to baseline, and sometimes an off-response is present at the cessation of the stimulus (Fig. 20, inset a). The $\mu\text{V}/\log I$ -function has a sigmoid shape (Fig. 20); and the visual threshold sensitivity, defined as the number of photons required to evoke a 5 μV response at peak wavelength ($\lambda = 495 \text{ nm}$), is $2.1 \cdot 10^8 \text{ quanta} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ (Fig. 20).

Light-exposed eye (LE)

Electroretinograms were immediately recorded after light exposure according to Table 1 and yielded weak responses in all cases except in the 117 lux light exposure (further commented below).

117 lux (exp. 2): Using the 5 μV response as the sensitivity criterion, a 10-min light exposure at this intensity prior to the experiment gave a response at a radiant flux of $5 \cdot 10^{10} \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$, i.e. about 2 log units above the ERG visual threshold of the DA eye. The response increased over 4 log units to a peak amplitude at 140 μV , and the curve is sigmoid. A slight DA occurred during the first 1.5 h, appearing as a rise in sensitivity (0.5 log units) before it ceased. The ERG wave form is similar to the DA eye.

If the light exposure time is increased to 1 h, a 5 μV response is obtained only at the highest stimulus intensity available ($3.4 \cdot 10^{13} \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$). No DA occurred during the 1.5-h test, and the ERG curve is similar to the DA criterion response curve.

1250 lux (exp. 3): No measurements were done.

2500 lux (exp. 4): Two animals were tested after a 1-h exposure. One had lost its sensitivity (on both eyes) and no recording trace could be obtained. The other gave a criterion response after 1.5-h test run and at an irradiance of $2.5 \cdot 10^{12} \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$, i.e. 4 log units above visual threshold (Fig. 20). The ERG of this LE eye seemed to have only an on-response, and at cessation of the light stimulus a very weak or no reaction was present. The response declined to baseline smoothly (AC recording).

Daylight (exp. 5): Irrespective of the exposure time, viz. 10 min or 4 h daylight (70 W/m^2) yielded a visual threshold at about $2 \cdot 10^{13} \text{ qu} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$. A further increase in stimulus irradiance destroyed the sensitivity and it can no longer be recorded.

Recovery experiments (exp. 6)

Some animals were dark-adapted (DA) for various periods of time prior to the measurements (Table 1, exp. no. 6) after different light exposures (Table 1, exps. 2-5) in order to study if a return to the visual threshold of the DA eye was possible. As a criterion the irradiance necessary to evoke a 5 μV response at peak wavelength, $\lambda = 495 \text{ nm}$, was used. This gave different results (Fig. 21), which can be summarized as follows:

117 lux: A 1-h exposure followed by 1 h of DA did not increase sensitivity, whereas a 10-min exposure followed by 12 h of DA restored the sensitivity to the ERG visual threshold of the DA eye (Fig. 21). The shape of the ERG curve is similar to the DA eyes. This is also true for the $\mu\text{V}/\log I$ curve.

1250 lux: Dark adaptations for different times, 6, 12, and 24 h, resulted in threshold sensitivities that cannot be correlated with the DA times (Fig. 20), and the results are dispersed around 2.5 log units (Fig. 21). The highest sensitivity in this group of adaptations was about 2 log units above the DA visual threshold. The $\mu\text{V}/\log I$ functions are sigmoid and the amplitudes saturate at about, or below, 170 μV . The ERG wave forms are similar to the DA eyes, except for the most insensitive preparation, which gave a slow return to baseline (Fig. 20, inset b). This was typical of preparations with sensitivities less than about $10^{11} \text{ qu}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ (see below). Noteworthy is, however, that all preparations were characterized by a hypersensitivity to strong light stimuli.

These radically depressed sensitivity and it could no longer be recorded during the experiment.

2500 lux: Compared with the previous experiment (1250 lux), similar results were obtained, i.e. no correlation between DA time and sensitivity could be obtained. The sensitivities were dispersed (around 2 log units) and the preparations were vulnerable to strong light stimuli, which destroyed the ERG. The visual threshold sensitivities were generally lower in these preparations and the highest sensitivity recorded was about 3 log units above that of the DA eye (Fig. 21). Peak amplitudes never exceeded 200 μ V and generally they were lower (Fig. 20). The preparations with the highest sensitivities yielded typical DA ERG curves, but those with a threshold sensitivity about 10^{11} qu $\cdot$ cm $^{-2}\cdot$ s $^{-1}$, or lower, resulted in slow ERG typical of the LE eyes.

Daylight: No responses could be recorded even after 5 days of dark adaptation.

DISCUSSION

Recently, the compound eye of Cirolana borealis was characterized functionally by the use of the electroretinogram, and this showed that the eyes possess a high visual threshold sensitivity (Lindström & Nilsson, 1982). It has also been shown that daylight exposures to the Cirolana eye alter the morphology of the photoreceptor membranes (Nilsson, 1982). The present results

extend these investigations by correlating the function and the morphological appearance of the eye at defined light exposures and at recovery. Compared with a dark-adapted eye, any light produces changes in the retinula cell morphology that includes the arrangement of the folds bearing the microvilli, the microvilli, the smooth endoplasmic reticulum (smooth ER), the Golgi apparatus, the mitochondria and the pigment granules. It should be noted that lysosome-like bodies are rarely seen in any case, and thus these are not likely to be involved in membrane renewal or breakdown processes as is the case in a number of arthropods (Blest, 1980; Eguchi & Waterman, 1967, 1976; Hafner et al., 1980; Nemanic, 1975; White et al., 1980).

Light induced a disordered arrangement of the cytoplasmic folds bearing the microvilli. It caused shortening of the microvilli and in severe cases these became disrupted. The increased distension of the smooth ER and number of Golgi complexes were also observed. And pigment moved from a position outside the rhabdom into the rhabdom space.

These morphological changes are interpreted as a retinal dystrophy, for they are reflected in the electroretinogram recordings as a loss of eye sensitivity and the changes cannot be restored to the condition of the dark-adapted eye (see below). The responses saturate at low amplitudes or they are totally depressed, and the curves of the ERG have a slow time course. A similar correlate, between the morphological appearance of the retina and its function, is present in light-exposed rat eye (Dowling & Wald, 1960; Noell et al., 1966), rabbit

(Lawwill, 1973a; Kuwabara, 1970) and in the monkey (Lawwill, 1973b), and also in crayfishes kept in prolonged darkness (Eguchi, 1965). If the light threshold for damage in the vertebrates mentioned above are compared with Cirolana, it will be found that the rat eye (Noell et al., 1966) and the Cirolana eye express similar sensitivities to light, whereas the threshold for the rabbit and the monkey is considerably higher (about 500 times). This is probably best explained by the different modes of living. Both the rat (nocturnal) and Cirolana normally live in dim light, which is in contrast to the diurnal mode of living in the other two species.

The results of different light exposures show that the eyes exposed to 117 lux (0.47 W/m^2 , exp. 2) react electrophysiologically very similar, although the sensitivity is slightly depressed as compared with the dark-adapted eye. The cytological changes are moderate with the exception of the double-membraned vesicles discussed below. Exposures for different times to light of 2500 lux or above gave little or no ERG response.

The structural changes imposed on the eye at higher illumination continue towards a greater disruption of the microvilli together with the folds that they are attached to. Frequently, membrane whorls are found and an increased number of irregularly shaped single-membraned vesicles originating from the distended smooth ER are present. The increase also involves the number and size of the Golgi complexes.

Disruption of photoreceptor membranes and whorl formation, caused by excessive light, is a well-described phenomenon in both arthropods (Behrens & Krebs, 1976; Blest & Day, 1978;

Loew, 1976; Meyer-Rochow, 1981; Nilsson, 1982; Tuurala & Lehtinen, 1971) and vertebrates (Kuwabara, 1970; Lawwill et al., 1980).

Similar effects are also caused by exposure of the eyes to prolonged darkness (Bloom & Atwood, 1981; Edwards, 1969; Eguchi & Waterman, 1966, 1979; Roach & Wiersma, 1974; Welsh, 1977), by a vitamin-A deficient diet (Carlson et al., 1969; Dowling & Gibbons, 1961; Yang et al., 1978), or by temperature (Meyer-Rochow & Tiang, 1979).

That membrane changes, in light and darkness, are introduced by inadequate fixation procedures is a legitimate concern (Kabuta et al., 1968; Williams, 1980; White & Michaud, 1981). These effects have, however, been considered and a suitable fixation procedure for the Cirolana eye is used (Nilsson, 1982).

The occurrence of a large number of distended cisternae of the smooth ER in the nuclei region of the reticular cells is similar to the case in the crayfish, Procambarus (Tsutsumi et al., 1981). In the latter animal, however, the greatest number of cisternae are found in the dark-adapted eyes in contrast to the light-exposed eyes in Cirolana.

Based on the occurrence of the distended ER in light-exposed eyes and on their location in the nuclear region of the cell, they cannot be regarded as subrhabdomeric cisternae (insects: reviewed, by Autrum, 1981; crustaceans: Eguchi & Waterman, 1967; Nässel & Waterman, 1979; Limulus: Behrens & Krebs, 1976). Rather, they may be interpreted as the visual proof of a disordered cell function, since distended ER is a typical feature of pathological cells (David, 1978; Trump et al., 1978). This also includes the

increased number (Ontell, 1975) of the hypertrophied Golgi complexes, at least when considering light levels above 117 lux. At this lower illumination, however, the increased number of Golgi complexes, and the associated large vesicles, may indicate a normal role in the membrane turnover process. A similar situation is also suggested by Stowe (1980) to be valid for the turnover mechanism in the crab eye, although no definite proof was found.

Attempts to restore the DA sensitivity and morphology of the eye to the level of the dark-adapted eye have been made. Only in the case of eyes subjected to 117 lux did they recover both functionally and structurally and even show pigment migration. The other recovery experiments in which the eye had been subjected to higher illumination before recovery did not elicit any signals or show structural restoration. Two findings deserve to be noted in this context. Exposure to 1250 lux or more initiates a structural derangement, which continues in the dark and eventually leads to a total breakdown. Thus, darkness does not trigger an immediate renewal of the membranes. A similar situation is also found in other crustacean eyes (Loew, 1976; Meyer-Rochow, 1981; Meyer-Rochow & Tiang, 1981).

Within the range of illumination of which the eye is not injured we found that even very short exposure times (10 min) needed 12 h of recovery to reach the values of the dark-adapted eye. This type of eye apparently has a very slow metabolism. This conclusion is supported by the finding that the regeneration rate of visual pigment is very slow in the deep-water-frequenting crustacean Nephrops (Loew, 1976).

Interesting also in this context is that the presence of light is not necessarily important (at least within a rather long period of time) to maintain normal function of the eye. This is evidenced by the fact that even after 2 months in total darkness the eye responds electrophysiologically quite normally when compared with ordinary dark-adapted eyes, and the visual threshold is very high (Lindström & Nilsson, 1982).

A characteristic type of double-membraned vesicles was found to occur in association with, or close to, the microvilli. They were infrequent in the dark-adapted eyes, but increased appreciably in eyes exposed to light, both below and above the damage threshold levels. A thorough analysis of the appearance of the vesicles, their morphology and connections with the microvilli revealed a new way, previously undescribed, by which reticular cells disintegrate microvilli. The process is thought to occur in several steps, which are illustrated in Fig. 21.

(1) The first step involves a bulging in of the membranes of two or more contiguous microvilli (2), and a new neck of the shortened microvilli is formed. (3) A similar invagination of the microvillar membrane occurs basally. (4) The next step involves, hypothetically, a closure of the membranes involved (this closure seems to first occur basally) and the double-membraned residual vesicle is formed. (5) The original basal extracellular space is released as a coated single-membraned vesicle. (6) The cytoplasm of the original microvilli ends joins that of the reticular cell. Various intermediate steps in the process are seen in Figs. 4, 9, 10, 14.

Different vesicle sizes are caused by (1) different lengths of the removed microvillar portions; (2) the various number of microvilli that partition in vesiculation; (3) several vesicles may coalesce to form membrane whorls (Fig. 11). The distorted wavy form of the microvillar lamellae, which is seen in light microscopical sections of light-exposed eyes, is an expression of irregularities in the microvillar lengths caused by vesiculation.

This mechanism could occur also in other species. Double-membraned vesicles seem to be present also in the Limulus eye (Behrens & Krebs, 1976). They could favour an interpretation of a similar mechanism for microvillar destruction along the lines outlined here. The coated vesicles, which are normally associated with membrane turnover and breakdown (Blest, 1980; Blest et al., 1978; Eguchi & Waterman, 1976; Stowe, 1980; White, 1967, 1968), seem to play a minor role, at least in the breakdown process, in the Cirolana eye, although these vesicles are still present.

Nothing is known at present of the future fate of the degraded microvillar membranes or how the synthesis of new photoreceptor membranes are accomplished.

The circumferential "tight junction"-like connections between the microvilli have their equivalents in the lateral eyes of Limulus (Behrens & Krebs, 1976; Lasansky, 1967) and in the cephalopod eye (Cohen, 1973; Moody & Robertson, 1960; Yamamoto et al., 1965). In Cirolana, an obvious objection to their presence is that they are fixation artifacts introduced by the fixative or by, e.g. osmosis (Falck et al., 1981; Karlsson & Schultz, 1965; Schultz & Karlsson, 1965). This would mean that a small extracellular space

could be present, i.e. a gap junction. At present, however, the true nature of the apposed membranes and the functional implications are unsolved.

In conclusion, our investigation has shown that the Cirolana eye is functionally adapted to low light intensities. This adaptation has evolved so far that exposure to moderate to high illumination is fatal to the eye and presumably also to the animal. The effects of a harmful initial exposure is continued in the dark and recovery is extremely slow. The described mechanism for microvillar membrane destruction is new among arthropods.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Fig. 1. Calibrated spectral irradiance, in absolute units ($\text{W}\cdot\text{m}^{-2}\cdot\text{nm}^{-1}$), for the broad spectrum white light that was used in the light-exposure experiments of the Cirolana compound eye. The calculated irradiances are measured over the range 390-690 nm. A = illuminance 2500 lux (1250 lux has the same spectral distribution), B = illuminance 117 lux. Abscissa: Wavelengths (λ) in nm. Ordinate: Irradiance ($\text{W}\cdot\text{m}^{-2}\cdot\text{nm}^{-1}$) of the exposure light.

Fig. 2. Schematic drawings (simplified), of cross sections, of the fused distal (a) and proximal open (b) rhabdom portions of the compound eye of Cirolana borealis. The rhabdom comprises seven rhabdomeres. DP = distal pigment cell sheet, Mv = microvillar lamellae of a rhabdomere, R = retinula cell, RP = reflecting pigment cell.

Fig. 3. Portion of a rhabdomere from a dark-adapted compound eye of Cirolana borealis. The well-organized microvilli (Mv) project, at right angles, from a retinula cell (RC) fold (asterisk), which projects into the rhabdom space. Large black arrow = a thin sheet of retinula cell cytoplasm that surrounds the rhabdom, small black arrows = pigment granules, white arrow = the region where opposing microvilli meet.

Scale: 2 μm , 5000X

Inset: Electron-dense deposits between the tips of opposing microvilli. Scale: 0.1 μm , 34,490X

Fig. 4. Microvillar bases of a dark-adapted eye. Note membrane vesicles, which are present and connected to a microvillus (asterisks) and also the thin necks (arrowheads) from which each microvillus originates. Mv = microvilli, S = single-membraned vesicle, black arrows = small extracellular spaces between the microvillar bases, open arrow = closed microvillar bases caused by an oblique section, white arrow = coated vesicle. Scale: 0.1 μm , 59,400X

Inset: Closed microvillar bases (open arrow) and the origin of a microvillus through a thin neck (arrowhead). Scale: 0.1 μm , 19,510X

Fig. 5. Five-layered appearance ("tight junction"-like) of adjoining microvilli (arrow). Scale: 0.1 μm . 126,250X

Fig. 6. Cross-sectioned microvilli (dark-adapted eye), which are hexagonally packed. Small arrows = thin extracellular channels between adjoining microvilli, large arrow = axial filament, which seems to be connected to the cell membrane. Scale: 0.1 μm , 77,550X

Fig. 7. The retinula cell cytoplasm of a dark-adapted eye. P = pigment granulae, arrows = smooth endoplasmic reticulum (ER), asterisks = distended ER. Scale: 0.2 μm , 47,000X

Inset: Golgi apparatus. Scale: 0.1 μm , 61,100X

Fig. 8. Part of a rhabdomere of an eye exposed to white light, 117 lux ($0.47 \text{ W}\cdot\text{m}^{-2}$) for 60 min. Note swollen and irregularly shaped microvilli (Mv), vesicle formation at the microvillar bases and retinula cell pigment granules in the rhabdom space. (Compare with Fig. 3.) Scale: 2 μm , 9400X

Fig. 9. Part of a rhabdomere of an eye exposed to white light, 2500 lux ($9.8 \text{ W}\cdot\text{m}^{-2}$) for 60 min. Note the large number of various sized vesicles. Some are seen connected to the microvilli (arrow) and others are free in the cytoplasm of the reticular cell fold. Scale: $1 \mu\text{m}$, 9460X

Fig. 10. A double-membraned vesicle still connected to the base of a microvillus. The eye was exposed to 117 lux ($0.47 \text{ W}\cdot\text{m}^{-2}$) for 60 min. Note the five-layered appearance of adjoining membranes and the presence of a fuzzy coat on both sides of the membrane (arrows). Scale: $0.1 \mu\text{m}$, 67,725X

Fig. 11. Membrane whorls of receptor membranes present at the microvillar bases (from an eye exposed to daylight). Scale: $1 \mu\text{m}$, 11,375X

Fig. 12. Golgi complexes in the retinula cell cytoplasm of an eye exposed to white light for 60 min ($117 \text{ lux}/0.47 \text{ W}\cdot\text{m}^{-2}$), asterisks = large vesicles associated with the Golgi complex, arrows = Golgi cisternae and associated small vesicles (fragmented?). Scale: $1 \mu\text{m}$, 12,000X

Fig. 13. Light microscopical cross section of the medial portion of the rhabdom (slightly oblique) of a daylight exposed eye (4 h). Note disordered microvillar lamellae and pigment granules in the rhabdom space. Scale: $15 \mu\text{m}$, 248X

Fig. 14. Cross-sectioned microvilli of an eye exposed to 1250 lux ($4.9 \text{ W}\cdot\text{m}^{-2}$) followed by dark adaptation for 24 h prior to fixation. Note disrupted and dilated microvilli, but also some microvilli that are still adjoined. Scale: $0.1 \mu\text{m}$, 29,575X

Fig. 15. Cross section of the rhabdom of an eye exposed to daylight for 4 h. Note disrupted microvilli (Mv) and pigment granules in the cytoplasmic folds of the rhabdoms (arrow). Scale: $1 \mu\text{m}$, 5000X

Fig. 16. Microvillar lamellae of the Cirolana rhabdom. The eye was exposed to 117 lux ($0.47 \text{ W}\cdot\text{m}^{-2}$) for 10 min and then dark-adapted for 12 h before fixation. Note well-organized microvilli with but few disruptions (open arrow) and a decreased vesicle formation at the microvillar bases (small arrow). Asterisk = single-membraned vesicle, large arrows = pigment granules. Scale: $1 \mu\text{m}$, 9460X

Fig. 17. Disrupted microvilli (Mv) of the Cirolana rhabdom after white-light exposure of 1250 lux ($4.9 \text{ W}\cdot\text{m}^{-2}$) for 10 min followed by dark adaptation for 12 h prior to fixation; large arrow = retinula cell pigment granules in the rhabdom; small arrow = distended smooth ER. Scale: $2 \mu\text{m}$, 3550X

Fig. 18. The rhabdom of the Cirolana eye after 4 h of daylight exposure followed by dark adaptation for 5 days prior to fixation. Note totally disrupted rhabdom (RH). RC = retinula cell. Scale: $5 \mu\text{m}$, 1880X

Fig. 19. The retinula cell cytoplasm of the Cirolana eye after 4 h of daylight exposure followed by 5 days of dark adaptation prior to fixation. Note disrupted mitochondria (asterisks) and irregularly shaped vesicles (arrows). P = pigment granules.

Fig. 20. Response-intensity functions ($\mu\text{V}/\log I$) measured by the ERG for the compound eye of Cirolana borealis. The measurements were either performed on truly dark-adapted eyes (DA) or on eyes exposed to white light followed by various dark adaptation times prior to the recordings (1-6). (Each function represents measurements from one animal.) Note the strong depression of the peak amplitudes of the light-exposed eyes; stippled curves = white-light exposures with an illumination of 1250 lux ($4.9 \text{ W}\cdot\text{m}^{-2}$) for 10 min followed by various dark adaptation periods prior to the measurements. (1) DA 6 h (2) DA 12 h (3) DA 24 h. Broken curves = white-light exposures of 2500 lux ($9.8 \text{ W}\cdot\text{m}^{-2}$) for 10 min followed by dark adaptation for 12 h (4 and 5) and light exposure for 60 min followed by DA 12 h prior to the measurements.

Inset: Typical electroretinogram curves for the dark-adapted eye (a) and the light-exposed eye (b). The recordings were made immediately after adaptation and exposure, respectively.

Fig. 21. Electroretinogram (ERG) measurements of visual threshold sensitivities for different white-light exposures, which were followed by a dark recovery time prior to the measurements for the compound eye of Cirolana borealis. (Each point represents measurements from one animal.) Abscissa: Energy content of the white-light exposures ($\text{J}\cdot\text{m}^{-2}$). Ordinate: Irradiance ($\text{qu}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$) necessary to evoke a criterion response (5 μV at peak amplitude $\lambda = 495 \text{ nm}$). DA = mean sensitivity of the deeply dark-adapted eye ($n = 19$). * 117 lux, 10 min DA 0 h, ✱ 117 lux, 10 min DA 12 h, □ 117 lux 60 min DA 0 h, ▲ 117 lux 60 min DA 1 h, ■ 1250 lux 10 min DA 6-24 h, ☆ 2500 lux 10 min DA 12 h, ★ 2500 lux 60 min DA 2-24 h, ⬤ 2500 lux 60 min DA 0 h, ○ Daylight – no responses could be detected.

Fig. 22. Schematic drawing illustrating how microvilli of light-exposed eyes in Cirolana borealis are thought to be shortened. Double-membraned- (4) and single-membraned-coated (5) vesicles are formed at the microvillar bases. (For further explanation, see the text.) Mv = microvillus, A = hexagonally packed microvilli seen in a cross section.

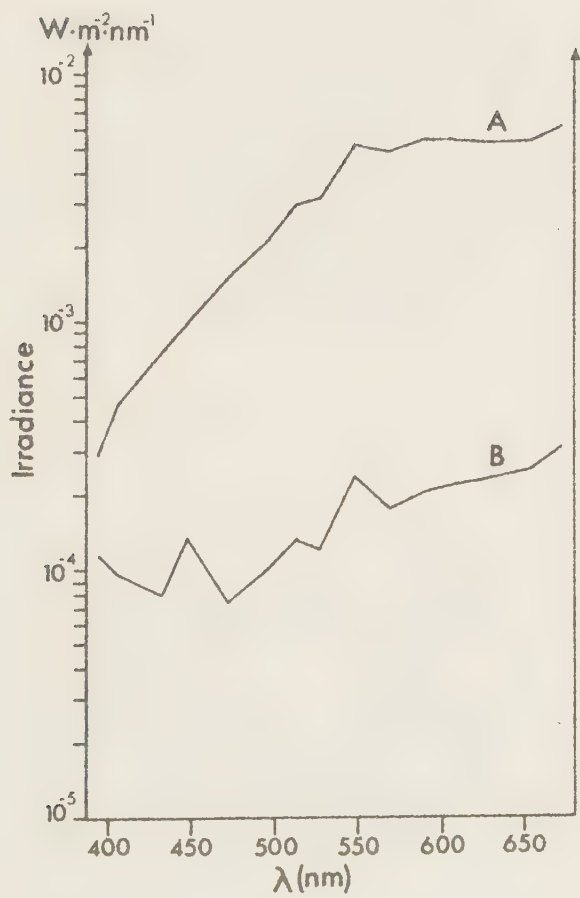
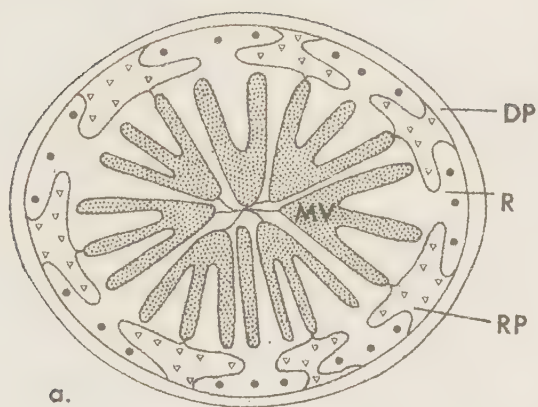


Fig. 1.

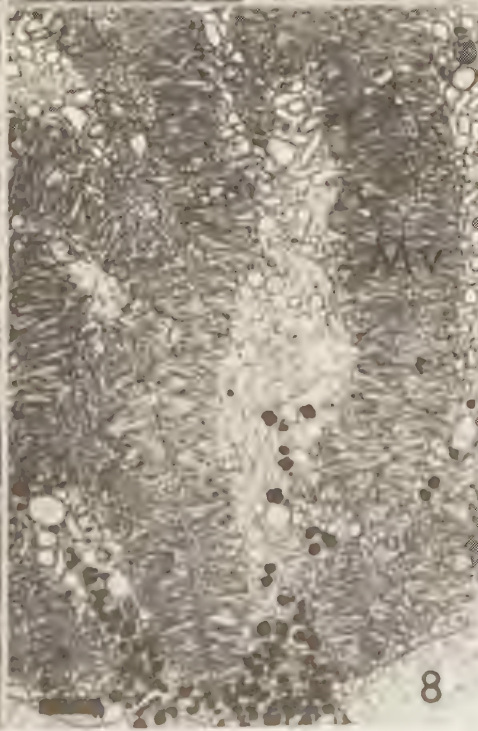
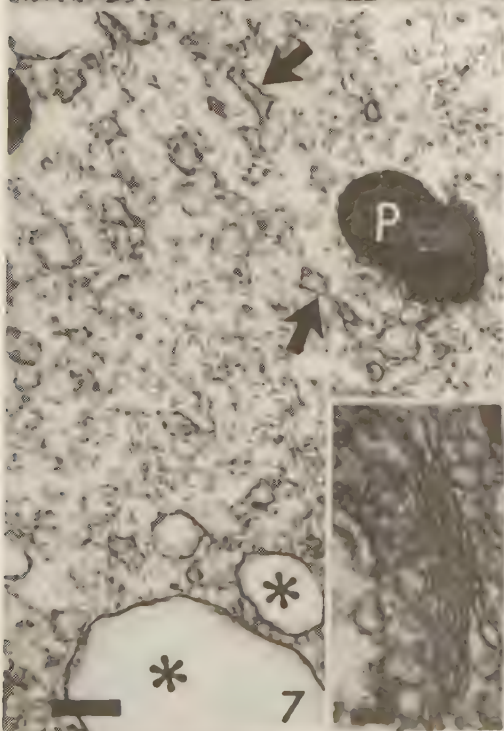
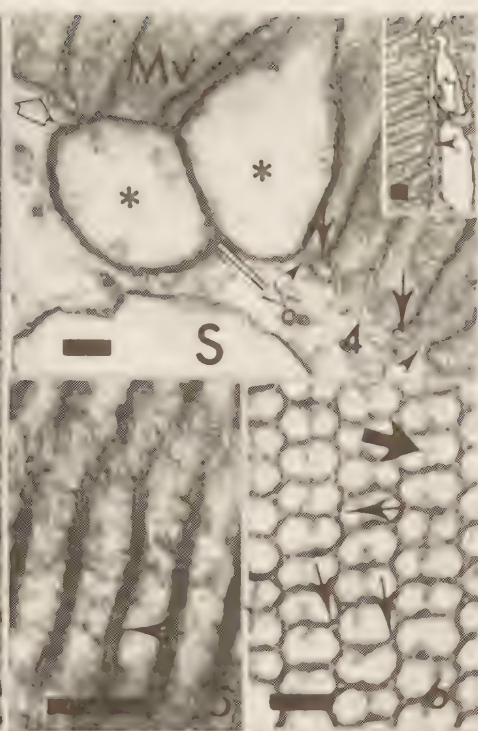


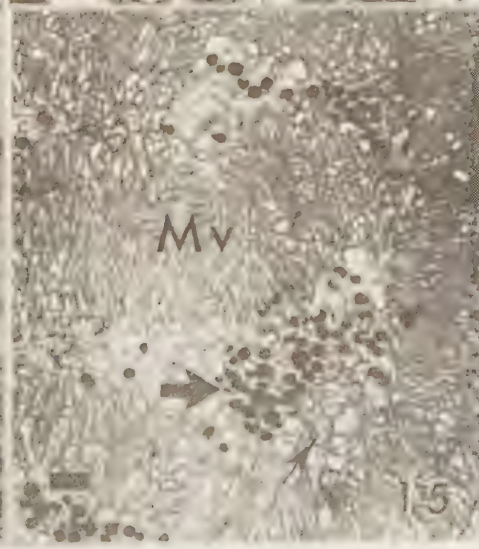
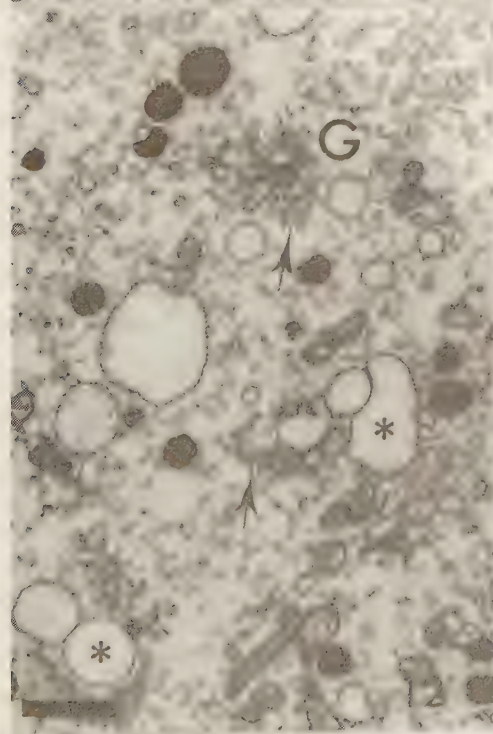
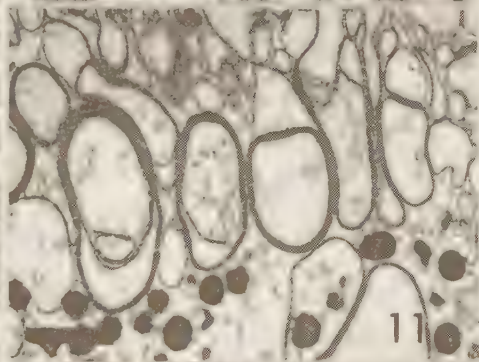
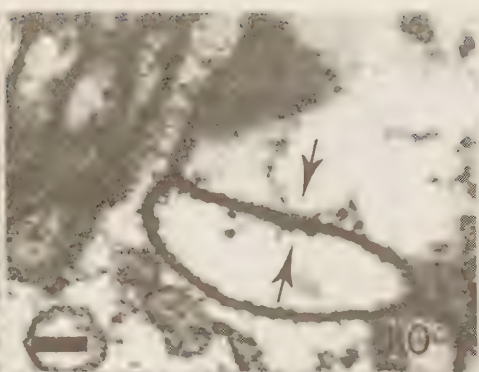
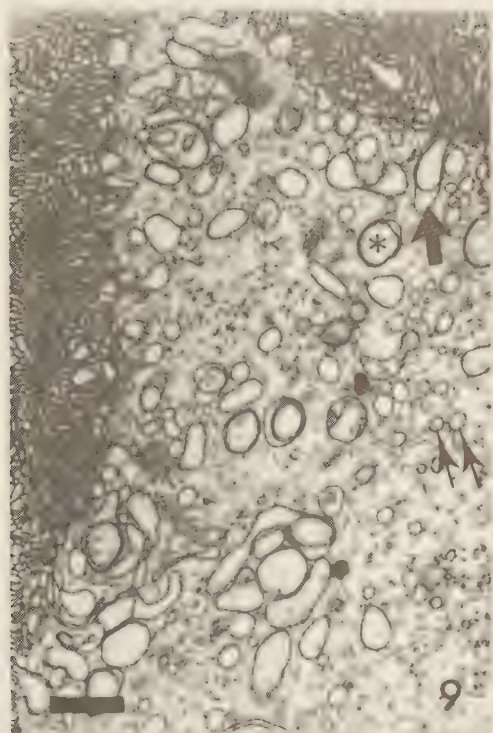
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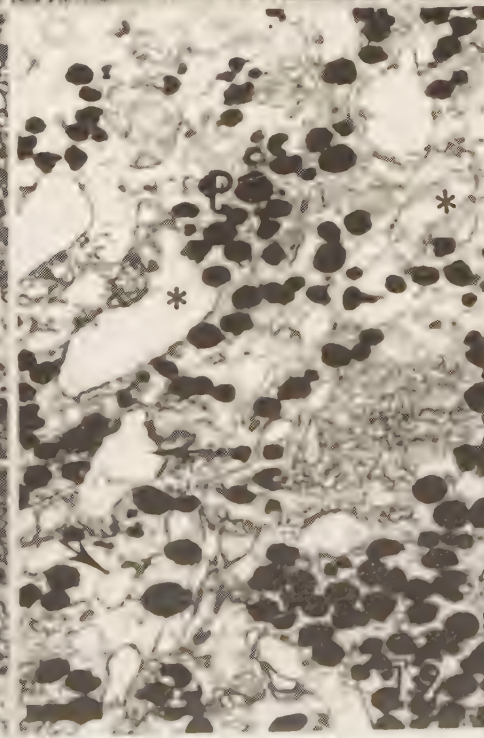


b.

Fig. 2.







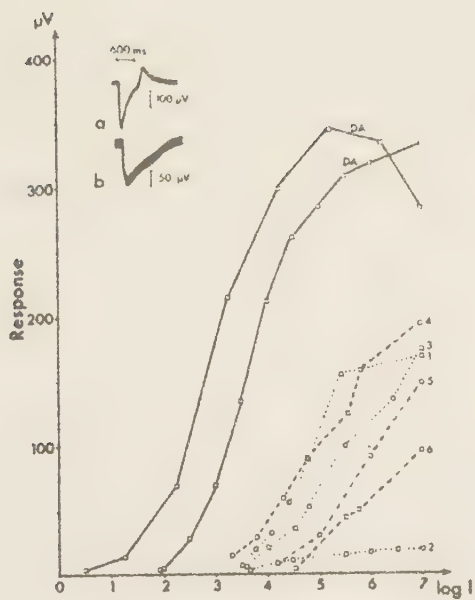


Fig. 20.

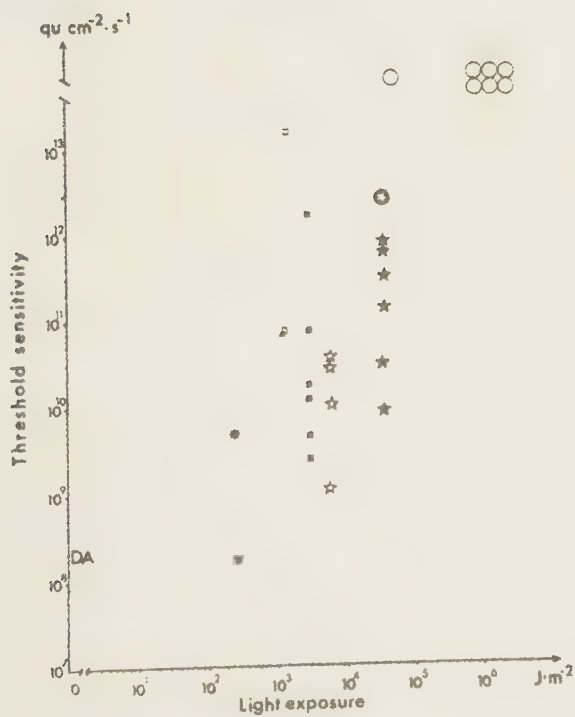


Fig. 21.

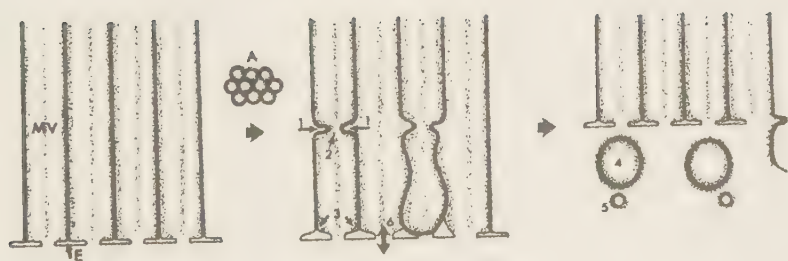


Fig. 22.

Light Exposure (LE)					Experiment no. 6 (exp. 6)		
Experiment no.	LE	Exposure time (min)	Number of animals		Recovery in darkness (h)	n_A	n_E
	Intensity lux ($\text{W}\cdot\text{m}^{-2}$)		n_A	n_E			
1	Dark-adapted	—	6	19*	—	—	—
2	117 (0.47)	10, 60	7	3	1, 12	2	2
3	1250 (4.9)	10, 60	—	—	6, 12, 24	4	7
4	2500 (9.8)	10, 60	2	1	2, 6, 12, 24	12	11
5	Daylight (70-136) (≥ 7500 lux)	10-240	13	4	24 h; 2, 3 and 5 days	4	6

Table 1. Light exposures of various intensities and durations of white light to the compound eyes of Cirolana borealis. In order to study recovery, some animals from experiments nos. 2-5 were left to dark-adapt after the light exposures and prior to the histological fixation and the electroretinogram (ERG) measurements (exp. no. 6). n_A = number of animals fixed for histology, n_E = number of animals killed for ERG measurements. Asterisk: see also Lindström & Nilsson (1982).

